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Concomitant use of medetomidine and vatinoxan in laboratory and client-owned dogs – Effects on drug absorption and disposition

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ACADEMIC DISSERTATION

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To my beloved ones.

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ABSTRACT

α_2-Adrenoceptor agonist drugs, such as medetomidine, are widely used in veterinary medicine for their sedative and analgesic effects. However, α_2-adrenoceptor agonists have undesirable cardiovascular effects, most notably vasoconstriction and bradycardia. In view of this, vatinoxan (previously known as MK-467 and L-659'066) a peripherally acting α_2-adrenoceptor antagonist has been investigated. Vatinoxan attenuates the negative cardiovascular effects of medetomidine in dogs while maintaining the sedative effects of the α_2-adrenoceptor agonist.

This thesis evaluated the effects of vatinoxan on the absorption and disposition of medetomidine and other selected drugs in laboratory and client-owned dogs following intramuscular administration; when the drugs were combined in the same syringe. The hypothesis was that vatinoxan would affect the plasma concentrations of concomitantly administered drugs, probably due to changes in tonus of the vasculature at the injection site. Furthermore, the distribution of vatinoxan and medetomidine into the central nervous system (CNS) was investigated. It was hypothesized that in dogs, vatinoxan would minimally enter the CNS, whereas the enantiomers of medetomidine would concentrate there.

A group of six laboratory beagles participated in the preclinical studies, and 57 client-owned dogs were recruited into the clinical study. A randomized, clinical study was designed to investigate whether vatinoxan attenuates the bradycardic impact of medetomidine in client-owned dogs given medetomidine-butorphanol intramuscularly for minor diagnostic procedures.

All analyses of drug concentrations were performed with liquid chromatography tandem mass spectrometry. Heart rates were monitored by auscultation and sedation was scored. Also, the degree of sedation was determined. It was hypothesized that vatinoxan would alleviate bradycardia without reducing the intensity of clinical sedation.

Vatinoxan initially increased plasma concentrations of concomitantly administered drugs. Improved cardiovascular performance in α_2-adrenoceptor agonist-medicated dogs probably affected the absorption via improved blood flow in the muscle. Furthermore, the distribution of enantiomers of medetomidine into the CNS appeared to be high whereas only about 2% of the vatinoxan plasma concentration was detected in the CNS. Vatinoxan ameliorated medetomidine-induced bradycardia, but adequate clinical sedation with a faster onset and shorter duration was achieved.

In conclusion, the increased absorption of concomitantly administered drugs by vatinoxan resulted in a quicker onset and shortened duration of the sedation. When vatinoxan was combined with medetomidine and butorphanol, it alleviated bradycardia and provided reliable sedation in healthy client-owned dogs.

LIST OF PUBLICATIONS

I. Kallio-Kujala IJ, Raekallio MR, Honkavaara J, Bennett RC Turunen H, Scheinin M, Hautajärvi H, Vainio O (2018) Peripheral alpha2-adrenoceptor antagonism affects the absorption of intramuscularly co-administered drugs. Veterinary Anaesthesia and Analgesia 2018;45:405-413

II. Honkavaara JM, Raekallio MR, Syrjä PM, Pypendop BH, Knych HK, Kallio-Kujala IJ, Vainio OM (2019) Central nervous system distribution of dexmedetomidine, levomedetomidine and MK-467, a peripherally acting α_2-adrenoceptor antagonist, after simultaneous intravenous administration in dogs. Veterinary Anaesthesia and Analgesia (in press). doi: 10.1016/j.vaa.2019.07.004

III. Kallio-Kujala IJ, Turunen HA, Raekallio MR, Honkavaara J, Salla K, Casoni D, Hautajärvi H, Vainio O. Peripherally acting α-adrenoceptor antagonist MK-467 with intramuscular medetomidine and butorphanol in dogs: A prospective, randomised, clinical trial. The Veterinary Journal 2018;240:22-26.

The publications are referred to in the text by their roman numerals (I-III).

ABBREVIATIONS

ABC	ATP-binding cassette, transporter superfamily
AUC	Area under time response curve
BBB	Blood-brain barrier
BCRP	Breast cancer resistance protein (also known as ABCG2)
BPM	Beats per minute
BSCB	Blood-spinal cord barrier
CL	Clearance
C_{max}	Maximum drug concentration in plasma
CNS	Central nervous system
CO	Cardiac output
EDTA	Ethylenediaminetetraacetic acid
HR	Heart rate
IM	Intramuscular
IV	Intravenous
MAC	Minimum alveolar concentration
MAP	Mean arterial pressure
MATE	Multidrug and toxin extrusion transporter
MDR	Multidrug resistance
MRP	Multidrug resistance protein
OAT	Organic anion transporter
OCT	Organic cation transporter
P-gp	P-glycoprotein (also known as ABCB1)
pK_a	The degree of ionization
SD	Standard deviation
SDS	Simple descriptive score, in this thesis used for assessing the sedation
SLC	Solute carriers, transporter superfamily
SVR	Systemic vascular resistance

T½	Half life
T_{max}	Time to Cmax
VAS	Visual analogue scale
V_z	Volume of distribution
QC	Quality control

1 INTRODUCTION

Sufficient delivery of a drug into the brain is essential when the target of the drug molecule is the central nervous system (CNS), as is the case with sedatives and anaesthetic drugs. The CNS is protected by blood-brain barrier (BBB) and blood-spinal cord barrier (BSCB): these physiological barriers provide a stable environment for neural function and protect the CNS from circulating neurotransmitters and xenobiotic substance. The challenge for a drug molecule is to pass these barriers. In addition to physiological properties of these barriers, the size and hydrophilicity of the molecule affects its ability to enter the CNS.

α_2-Adrenoceptor agonists are widely used in veterinary medicine for their sedative and anxiolytic properties, but their undesired cardiovascular effects, such as bradycardia and decreased cardiac output (CO), limit their usefulness. Medetomidine is a selective, potent and efficacious α_2-adrenoceptor agonist that produces sedation and antinociception by stimulating α_2-adrenoceptors in the central nervous system (Doze et al. 1989, Maze & Fujinaga 2000). Vasoconstriction is caused by activation of α_2-adrenoceptors within vascular smooth muscle (Clough & Hatton 1981, Horn et al. 1982). Through a baroreceptor-mediated reflex, vasoconstriction leads to bradycardia which is responsible for a dramatic decrease in CO and oxygen delivery (Bloor et al. 1992, Pypendop 1998).

In view of this, the ability of vatinoxan to attenuate the negative cardiovascular effects of dexmedetomidine and medetomidine in dogs has been investigated (Pagel et al. 1998, Enouri et al. 2008, Honkavaara et al. 2008, 2011). Vatinoxan predominantly blocks peripherally located α_2-adrenoceptors due its limited ability to cross the BBB, as demonstrated in rats and marmosets (Clineschmidt et al. 1988). Therefore, it attenuates the initial vasoconstriction produced by α_2-adrenoceptor agonist and the following haemodynamic disturbances in dogs (Pagel et al. 1998, Enouri et al. 2008, Honkavaara et al. 2011), whereas the CNS effects of α_2-agonists (sedation) are not decreased substantially (Pagel et al. 1998, Enouri et al 2008, Honkavaara et al 2008).

Vatinoxan alters the disposition of α_2-adrenoceptor agonists, an effect that probably results from improvement of perfusion as demonstrated after intravenous (IV) administration to dogs (Honkavaara et al. 2012) but also inhibition of local vasoconstriction after intramuscular (IM) co-administration (Restitutti et al. 2017).

The studies reported in this thesis were designed to test the following hypotheses: vatinoxan would alleviate medetomidine-induced bradycardia in healthy research and client-owned dogs without alleviating the desired central effects (Study I and III) as demonstrated earlier in dogs and many other species, and that vatinoxan would show high peripheral selectivity in dogs (Study II), as previously demonstrated in marmosets and rats (Clineschmidt et al. 1988). An additional, hypothesis was that the accelerated IM absorption of concomitantly given drugs caused by vatinoxan might quicken the onset and conversely, shorten the duration of sedation (Studies I and III).

2 REVIEW OF THE LITERATURE

2.1 Background of receptor agonism and antagonism

The role of receptors as the target of drug action has guided the understanding of drug action and drug discovery since the beginning of 20th century (Langley 1901, 1905). Langley (1905) described a receptive substance (acetylcholine) of a skeletal muscle that was responsible for a cascade (binding at the postsynaptic membrane of the neuromuscular junction) that resulted in a physiological response (skeletal muscle contraction). The concept of drug agonism and antagonism was described by Gaddum (1937), who introduced a model of a competitive receptor binding of two ligands. In the past 60 years, the current understanding of receptors has been built on by the work of multiple pharmacologists (Rang 2006).

The structure of the ligand, a substance that forms a complex with a biomolecule, determines whether it works as an agonist or antagonist at a receptor (Matsui et al. 1989, Khan et al. 1999). To be able to bind a ligand, a receptor must have a stereoscopic counterbalancing structure within it that matches the ligand (Matsui et al. 1989). As postulated by receptor theory, a receptor must possess structural and steric specificity, and the receptors have to be finite in number and saturable. Additionally, a receptor must have a high affinity for its endogenous ligand at physiological concentrations, and the binding of the endogenous ligand should lead to a recognizable chemical event in the body (Rang 2006).

An agonist is a molecule that binds to receptor, resulting into an activation of that specific receptor. This activation produces a biological response. An antagonist, on the other hand, blocks the agonist from activating the receptor. Where a full agonist will always generate a maximal response in the receptor, a partial agonist generates a submaximal response. Some partial agonists, such as buprenorphine for μ-opioid receptors (Dum & Herz 1981), have a high affinity for the receptor and therefore can act as an antagonist in the presence of a full agonist, as the partial agonist can prevent full agonist-receptor bonding.

An antagonist, as opposed to an agonist, produces no recognizable events in the receptor, but prevents other ligands from binding to the receptor (Colquhoun 1973). If two agonists bind to the same receptor, they can be antagonised by the same antagonist. Competitive antagonism can be either reversible or irreversible. Reversible antagonism can be

overridden with increased agonist doses, whereas irreversible antagonism cannot be overridden when high doses of agonist are present. A drug can also work as a ligand at multiple receptors, and therefore act as an agonist and antagonist simultaneously: for an example, butorphanol is both a μ-antagonist and κ-agonist, as it has affinity for both μ- and κ- opioid receptors (Commiskey et al. 2005).

To simplify the relationships, if the efficacy of a full agonist is equal to 1, an antagonist should be perceived as 0, and a partial antagonist valued between 0 and 1 (Ross 1996). The α_2-adrenoceptor agonists (such as dexmedetomidine) used in veterinary medicine are selective, potent agonists for α_2-adrenoceptors both in the central and peripheral nervous system (Doze et al. 1989, Maze & Fujinaga 2000) whereas atipamezole is a highly selective α_2-adrenoceptor antagonist (Virtanen et al. 1989).

2.2 α-adrenoceptors

Initially, adrenoceptors were divided into two main classes: α and β (Ahlquist 1948). However, currently, α-adrenoceptors are divided into two main groups (α_1 and α_2) and each is further divided into three subtypes. α_1-subtypes (α_{1A}, α_{1B}, α_{1D}) and α_2-subtypes (α_{2A}, α_{2B}, α_{2C}) (Bylund 1988, Bylund et al 1994). α-adrenoceptors consist of a long chain of amino acids, that form both hydrophilic and hydrophobic areas, traversing the cell membrane in total seven times. These seven hydrophobic segments consist of 20-25 amino acids (Matsui et al. 1989).

Adrenoceptors are a part of the autonomic nervous system and the endogenous ligands for these receptors are catecholamines, such as noradrenaline. Adrenoceptors are G-protein-linked cell membrane receptors that are distributed within the CNS and peripheral tissues (Gilman 1987). They are excitable proteins which selectively react to both endogenous hormones and externally given chemicals to initiate a cascade resulting in a physiological effect (Matsui et al. 1989, Khan et al. 1999).

α_{2A}-adrenoceptors mediate sedation and antinociception (Hunter et al. 1997, Lakhlani et al. 1997, Stone et al. 1997) as well as anesthetic-sparing effects (Lakhlani et al. 1997). The *locus coeruleus* is the largest noradrenergic cell group in the brain and located bilaterally in the upper brain stem. It participates in regulating wakefulness or consciousness and is the most important site for the hypnotic effects of α_2-adrenoceptor agonists (Scheinin & Schwinn 1992). In general, presynaptic α_2-adrenoceptors, which are primarily of the α_{2A}-subtype

(Brede et al. 2003), modulate neurotransmitter release, such as noradrenaline, from nerve endings via negative feedback mechanism (Langer 1974, Ruffolo 1985) (Figure 1). Current α_2-adrenoceptor agonists used in veterinary medicine for provision of sedation are not selective to any specific α_2-subtypes.

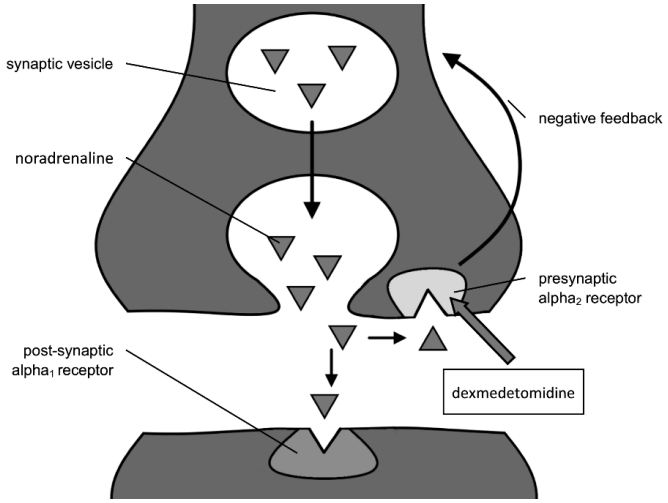

Figure 1. Presynaptic effect of α_2-receptors on the CNS. Most of the presynaptic α_2-receptors are located in the *locus coeruleus*. The endogenous ligand noradrenaline and exogenous α_2-adrenoceptor agonists, such as dexmedetomidine, affect the same receptors.

The location of the adrenoceptors within the sympathetic nerve supply, has functional significance. α-adrenoceptors mediate almost exclusively the effects of the sympathetic portion of the autonomic nervous system peripherally. Adrenoceptors are activated by neurotransmitters released from the terminal of the sympathetic nerves or they can be stimulated by catecholamines circulating in the body (Guimaraes & Moura, 2001). Hence, extra-junctional α-adrenoceptors are stimulated by circulating adrenaline and noradrenaline secreted from the adrenal medulla (Langer et al. 1981).

Post-synaptic receptors that are located on the effector organ tissues or vessels and are usually of the α_1-subtype. Post-synaptic receptors that are immediately associated with the sympathetic nerve varicosity are called junctional receptors, whereas postsynaptic receptors without direct innervation, are called extra-junctional receptors (Ruffolo, 1985).

The biological effects of adrenoceptors may differ depending on their location, for example on nerves, blood vessels or parenchyma of various tissues (Ruffolo 1985, Guimaraes & Moura, 2001, Koshimizu et al. 2002). Furthermore, if there is more than one receptor subtype present in the same tissue, the effect of an agonist on the receptor is dependent on the relative prevalence of the different receptor populations (Guimaraes & Moura, 2001). The response in the end-organ is always dependent on the interactions of the α-adrenoceptors and catecholamines that are released from the adrenal gland and the sympathetic nerve endings (Bennett et al. 1998). Most of the sympathetic functions are controlled by α-adrenoceptors that are located near a sympathetic synapse, whereas receptors that lack innervation have an additional role. (Brede et al. 2003).

Smooth muscle in the vasculature, with the exception of pre-capillary sphincters, has broad α-adrenergic innervation (Pang 2001, Chiba & Yang 2003). The tone of the sympathetic nervous system is responsible for maintaining partial vascular smooth muscle contraction (Long & Kirby 2008). Both α_1- and α_2-adrenoceptors regulate vasomotor tone (Piascik et al. 1990). In genetically altered mice, α_{2B}-subtype is mainly responsible for mediating arterial vasoconstriction. Nevertheless, also α_{2A}- and α_{2C}-adrenoceptors mediate the vasoconstriction of smaller arteries (Chotani et al. 2004). Postsynaptic receptors that regulate basal vasomotor tone are mainly α_1-adrenoceptors, which are activated by the catecholamines released from adrenal medulla or from the sympathetic nerve endings (Langer et al. 1981, Ruffolo 1985, Docherty 2010). In general, α_1-adrenoceptors are junctional and are primarily responsible for vasoconstriction (Tanoue et al. 2003) since they stimulate the contraction of smooth muscle within the wall of arteries and arterioles (Guimaraes & Moura, 2001). α_1-adrenoceptors are located to receive direct messages from the vasomotor centre in the CNS, and therefore are the primary effectors of changes in centrally mediated sympathetic tone (Guimaraes & Moura, 2001). Peripheral α_2-adrenoceptors, that are typically extra-junctional (Yamaguchi & Kopin 1980, Langer et al. 1981), have a lesser role in systemic arterial vasoconstriction compared to α_1-receptors (Leech & Faber 1996), but regulate the vasoconstriction of smaller peripheral arteries (Chotani et al. 2004).

Venous vasculature also has extensive sympathetic innervation. Post-synaptic α_1- and α_2-adrenoceptors mediate the constriction of venous smooth muscle (Pang 2001). On the contrary to arterial circulation, venous α_2-adrenoceptors are predominantly junctional and α_1-adrenoceptors are primarily extra-junctional (Guimaraes & Moura, 2001). Therefore, the

most important receptor responsible for the regulation of the venoconstriction is the α_2-adrenoceptor. In the dog the cutaneous or splanchnic venous circulation have particularly dense α_2-receptor populations (Flavahan & Vanhoutte 1986, Guimaraes et al. 1987).

α_2-adrenoceptors take part in mediation of multiple functions in the body. α_{2A}-adrenoceptors have an important role on for an example glucose homeostasis and regulation of insulin secretion (Peterhoff et al. 2003, Fagerholm et al. 2004, 2008, Savontaus et al. 2008) as well as platelet aggregation (Scheinin & MacDonald 1989). α_{2A}-adrenoceptors receptors are responsible for mediating hypothermia (Hunter et al. 1996), hypotension and bradycardia (MacMillan et al. 1996, Altman et al 1999). Some of the physiological effects of different α_2-adrenoceptor subtypes are listed in Table 1.

Table 1. Some physiological effects mediated by α_2-adrenoceptor subtypes

Receptor subtype	Physiological response	Anatomical location	References
α_{2A}	Hypotensive, bradycardic action	Central sympatholysis	Altman et al. 1999, MacMillan et al. 1996
	Spinal antinociception	Spinal cord	Stone et al. 1997
	Sedation	Cerebral cortex Locus coeruleus	Hunter et al. 1997, Lakhalani et al. 1997
	Aggregation	Platelets	(Scheinin & MacDonald 1989)
	Arterial vasoconstriction	Arterial walls	MacMillan et al. 1996
	Thermoregulation	Hypothalamus	Hunter et al. 1997
	Glucose homeostasis, regulation of insulin secretion	Pancreatic β-cells	Peterhoff et al. 2003, Fagerholm et al. 2004, 2008, Savontaus et al. 2008
α_{2B}	Vasoconstriction	Vascular smooth muscle	Link et al. 1996, Makaritsis et al. 1999
α_{2C}	Spinal antinociception	Spinal cord (dorsal root ganglia)	Fairbanks et al. 2002
	Thermoregulation	Hypothalamus	Sallinen et al. 1997

In summary, as composed in the α-adrenoceptor review by Giovanniitti (2015) α_1-adrenoceptors are responsible for constriction of vascular smooth muscle, radial muscle of the eye and smooth muscle of the vas deferens. α_2-adrenoceptors, on the other hand, are responsible for centrally induced sedation via locus coeruleus and inhibition of norepinephrine release from presynaptic neurons. Additionally, α_2-adrenoceptors mediate centrally mediated pain modification through the dorsal horn and inhibition of insulin release (GIovanniitti et al 2015).

2.3 α_2-adrenoceptor agonists

α_2-adrenoceptor agonists are widely used in veterinary medicine for their sedative, analgesic and anxiolytic effects. This group of drugs has been in use in the veterinary field since the 1960s when xylazine was introduced for horses and cattle (Clarke and Hall, 1969). Later on, more novel drugs with a higher selectivity towards α_2-adrenoceptors have been developed. Nowadays multiple α_2-adrenoceptor agonists are available for veterinary use, such as xylazine, romifidine, clonidine, detomidine, medetomidine and dexmedetomidine. In small animal medicine, medetomidine and dexmedetomidine are currently the most frequently used α_2-adrenoceptor agonists, due to their greater affinity for α_2-adrenoceptors (α_2:α_1-ratio of 1620:1 with medetomidine, compared to α_2:α_1-ratio of 160:1 for xylazine) (Virtanen et al. 1988).

Sedative effects of α_2-adrenoceptor agonists result from inhibition of noradrenaline release from noradrenergic receptors. These receptors are located in the *nucleus locus coerulus*, and they are thought to be responsible for the controlling the sedative as well as antinociceptive effects of α_2-adrenoceptor agonists (Scheinin and Schwinn, 1992).

However, all α_2-adrenoceptor agonists have adverse effects mainly related to depression of the cardiovascular system. They induce vasoconstriction mediated via post-synaptic α_2-receptors located in vascular smooth muscle (Bloor et al. 1992, Flacke et al. 1993, Pypendop & Verstegen, 1998). Higher systemic vascular resistance occurs in this phase. Bradycardia is attributed partly to a reflex elevation on vagal tone because baroreceptors are stimulated by the elevated blood pressure, and persists after the hypertensive phase resulting in reduced cardiac output (Thurmon et al 1994: Sinclair et al 2003) and oxygen delivery (Doxey et al 1981, Savola 1989, Bloor et al 1992, Pypendop et al 1998).

In addition to central and cardiovascular effects, α_2-adrenoceptor agonists have vast effects on other body functions. Medetomidine inhibits insulin secretion resulting in hyperglycemia and increases growth hormone secretion. Additionally, it increases diuresis and reduces plasma concentrations of catecholamines similarly to other α_2-adrenoceptor agonists (Schenin et al. 1987).

2.3.1 Medetomidine and dexmedetomidine

Medetomidine (International Union of Pure and Applied Chemistry name [(RS)-4-[1-(2,3-dimethylphenyl)ethyl]-3H-imidazole] is a racemic mixture of two enantiomers, dexmedetomidine [(+)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole] and levomedetomidine [(-)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole]. Dexmedetomidine is the active component, whereas levomedetomidine is considered pharmacologically inactive (MacDonald et al. 1991, Kuusela et al. 2000, 2001). In canine plasma, medetomidine is bound to protein up to 85% (Salonen 1989). The elimination half-life was approximately 60 minutes after intravenous administration in dogs, whereas clearance was 1.3-1.8 l/kg/h (Salonen 1989, Kuusela et al. 2000).

(Dex)medetomidine is widely used in small animal veterinary medicine. It is a selective and efficacious agonist at α_2-adrenoceptors both in the central and peripheral nervous system (Doze et al. 1989, Maze & Fujinaga 2000) that produces sedation, muscle relaxation and antinociception in dogs (Vainio et al. 1989, Salonen et al. 1992, Grimm et al. 2000, Kuusela et al. 2001, van Oostrom et al. 2011). Dexmedetomidine induces dose-dependent sedation by decreasing the activity of noradrenergic receptors within the CNS (Rabin et al. 1996, Millan et al. 2000, Lähdesmäki et al. 2003). (Dex)medetomidine induces the depressant effects on the cardiovascular system typical of all α_2-adrenoceptor agonists, such as bradycardia, increased systemic vascular resistance, and decrease in oxygen delivery, as described earlier in the review.

Dexmedetomidine is commonly used as a part of an anaesthetic regimen because its administration potentiates the effects of other hypnotic and sedatives agents (Salonen 1989, Scheinin et al. 1993, Ben-Abraham et al. 2000, Kuusela et al. 2001). When used as a preanesthetic, dexmedetomidine decreases the minimum alveolar concentration (MAC) of halothane (Vickery et al. 1988, Bloor et al. 1992), isoflurane (Pascoe et al. 2006) and sevoflurane (Hector et al. 2017).

2.4 α_2-adrenoceptor antagonists

Reversal of the sedation caused by α_2-adrenoceptor agonists is often required for practical reasons after sedation of animals. α_2-adrenoceptor antagonists, such as yohimbine, tolazoline and atipamezole, can be used as reversal agents. Yohimbine, a compound that was extracted from a bark of a tree over 100 years ago, was the first α_2-adrenoceptor antagonist used in the field of veterinary medicine.

As described with α_2-adrenoceptor agonists earlier, similar development towards more selective α_2-adrenoceptor antagonists has been seen. Respectively, atipamezole has a α_2:α_1- selectivity ratio of 8526:1 compared to 40:1 to yohimbine and 27:1 to idanoxan (Virtanen et al. 1989).

2.4.1 Atipamezole

Atipamezole is a potent α_2-adrenoceptor antagonist that is widely used in veterinary medicine for reversal of both sedative and analgesic effects of α_2-adrenoceptor agonists. It affects both centrally and peripherally located α_2-receptors (Virtanen et al. 1989). It reverses the centrally-mediated effects of dexmedetomidine (Clarke & England 1989, Vainio 1990, Vähä-Vahe 1990, Granholm et al. 2007).

In addition to blocking of the central effects of α_2-adrenoceptor agonists, such as sedation, atipamezole has an effect on peripheral α_2-adrenoceptors (Virtanen et al. 1989), leading to the reversal of medetomidine-induced bradycardia (Vähä-Vahe 1990). However, transient hypotension may be seen, probably due to vasodilation (Vainio 1990). When higher atipamezole doses are used, greater increases in heart rate (HR) are observed (Vähä-Vahe 1990), but hypotension may also be detected (Vainio 1990). Atipamezole doses that were five or ten times the preceding dose of medetomidine (20, 40 or 80 µg/kg of medetomidine IM) given 30 minutes after medetomidine, significantly increased HR in dogs from 40-46 beats per minute to 67-95 beats per minute within 10 minutes. Additionally, mean arterial pressure (MAP) decreased within 3 minutes after atipamezole administration (Vainio 1990).

In dogs treated with medetomidine and vatinoxan, atipamezole can be used to reverse the sedative effects of medetomidine in the presence of vatinoxan without adverse cardiovascular effects. Atipamezole failed to increase cardiac index in dogs given medetomidine, while vatinoxan enhanced haemodynamic function (Turunen et al. 2019).

Atipamezole alters the elimination of medetomidine, as the administration of atipamezole intramuscularly reduces the area under curve (AUC) of medetomidine in medetomidine-sedated dogs (Salonen et al. 1995). Atipamezole, as well as medetomidine are metabolized in the liver through hydroxylation, but no metabolic interaction between these two drugs has been detected (Salonen et al. 1995). However, increased clearance of medetomidine in the presence of atipamezole has been thought to be a result from the restoration of hemodynamic function after atipamezole administration (Baratta et al. 2009).

2.4.2 Vatinoxan

Vatinoxan is a selective (α_2:α_1-ratio 105:1) peripheral α_2-adrenoceptor antagonist that poorly penetrates the BBB (Clineschmidt et al. 1988). Brain/plasma concentration ratios of 0.02 for marmosets and 0.05-0.09 for rats for vatinoxan have been reported. This finding has been speculated to result from the relatively low lipid solubility of vatinoxan (Clineschmidt et al. 1988). Hence, the α_2-agonist-induced sedation is not markedly decreased by vatinoxan, as demonstrated in dogs (Pagel et al. 1998, Enouri et al. 2008, Honkavaara et al. 2008, Restitutti et al. 2011) whereas the negative peripheral effects, such as hypertension and bradycardia, have been alleviated with both IV and IM administration of vatinoxan in this species (Pagel et al. 1998, Honkavaara et al. 2011, Rolfe et al. 2012, Restitutti et al. 2017). Elimination half-life for vatinoxan is approximately 40 minutes and clearance is 7.8 ± 3.4 ml/kg/minute in dogs (Honkavaara et al. 2012).

Generally, attenuation of the initial vasoconstriction is considered to be desirable because it alleviates the baroreflex-mediated bradycardia (Pypendop et al. 1998). Conversely, when the α_2-adrenoceptor agonist-induced vasoconstriction is ameliorated, the risk for hypotension increases. It was demonstrated in isoflurane-anaesthetized dogs that preoperative administration of medetomidine and vatinoxan provided cardiovascular stability during both the preanesthetic and perioperative period. While the effect of a medetomidine and vatinoxan combination on blood pressure was comparable to that produced by acepromazine-butorphanol when these drugs were used for premedication (Salla et al. 2014a). Acepromazine decreases arterial blood pressure through vasodilation mediated via α_1-adrenoceptors blockade (Ludders et al. 1983, Monteiro et al. 2007).

The clinically optimal dexmedetomidine:vatinoxan dose ratio seems to be dependent on the species and route of administration. In dogs, dexmedetomidine:vatinoxan ratios between

1:20-1:80 prevented the immediate cardiovascular effects of dexmedetomidine when vatinoxan was administered prior to IV dexmedetomidine (Pagel et al. 1998). Additionally, Honkavaara et al. (2011) reported that a dexmedetomidine:vatinoxan dose ratio of 1:50 (using dose ratios of 1:25, 1:50 or 1:75) seemed most appropriate for dogs when used IV in the same syringe. Restitutti et al. (2017) administered vatinoxan IM at three dose ratios concomitantly with medetomidine (corresponding dexmedetomidine:vatinoxan ratios of 1:20, 1:40 and 1:60), and initially with all three doses, the cardiovascular effects of medetomidine, such as decreased HR and cardiac output (CO), were demonstrated. However, with the two higher doses of vatinoxan, MAP transiently increased and then significantly decreased from the baseline (Restitutti et al. 2017).

2.5 Principles of drug absorption, distribution, metabolism and excretion (ADME)

There are multiple routes for drug administration, such as IM, IV, gastrointestinal, percutaneous, respiratory, and subcutaneous. In this thesis, the IM route is studied. The distribution of drugs, particularly vatinoxan, into the CNS is emphasized. Distribution to peripheral tissues depends on the physiochemical properties of the molecule, concentration gradient between the blood and tissue, the blood flow to the tissue and the affinity of the drug molecule for different tissue components. Since this thesis focuses on the absorption and distribution phases, the details of drug metabolism and elimination are not described.

2.5.1 Absorption

In order to move in order to enter and exit cells, drugs as well as other molecules must cross semipermeable lipid-based membranes. Lipid-soluble, nonpolar molecules are able to readily pass these membrane barriers. Additionally, membranes are more permeable to the nonionized forms of weak organic acids and bases. The degree of ionization (pK_a) defines what proportion of a drug is present in its nonionized form at a given pH. Briefly, lipid-soluble drugs are readily absorbed into the body, whereas hydrophilic drugs are absorbed more slowly (Becker & Reed 2012).

2.5.1.1 Intramuscular absorption

In addition to a drug's physical properties, absorption can be influenced by the physiological state of the animal, the administration site and certain chemical factors, such as inflammatory mediators. The physiological state of the animal can affect the absorption through vascular tone. Vasodilation increases, while, vasoconstriction delays the IM absorption. One way to either delay or increase the absorption phase is to alter the vascular tone of blood vessels at the injection site. Vasopressors added to local anaesthetic formulations prolong the absorption phase by causing vasoconstriction. For example, adrenaline is added to local anaesthetic solutions for this purpose (Becker & Reed 2012). The absorption of drug molecules into a tissue also depends on capillary density and the mass of the target tissue.

The IM route of administration has been widely used and studied in veterinary medicine due to its convenience during clinical work. This route is also used when it is not possible to obtain IV access without causing excessive stress and discomfort. In humans, the IM route is used when the IV route is not a viable option due to the low aqueous solubility of a drug or if the high peak concentrations after IV administration might cause unwanted effects (Zuidema et al. 1988).

Drug release after IM administration is affected by the molecular mass of the drug, its pK_a, co-solvents, a drug's water solubility and concentration, the depth of injection, muscle activity, blood supply at the injection site and body movement (Zuidema et al. 1988). Additionally, binding of the drug to the tissue plays an important role in the absorption phase (Sund & Schou 1964). One possible factor causing variation in IM absorption is the temperature of the injected solution. Administration of azaperone and ketamine to pigs, at temperatures of 7, 20 and 39°C, showed that animals became recumbent more rapidly following injection of the two warmer mixtures. Warmer injectates are believed to dilate the diameter of blood vessels in the muscle, leading to a more rapid absorption of the drugs (Clutton et al. 1998).

The activity and blood flow to a muscle (Self et al. 2009, Benet et al. 2011), perimuscular fat and intermuscular fascial planes (Sund & Schou 1964, Baxter & Evans 1973) affect the bioavailability of the drug. The rate absorption of drug molecules from the adipose tissue is slower than that from the muscle tissue, owing to the poor blood flow in the former tissue (Dundee et al. 1974, Pieters & Zuidema 1986). Resting muscle blood flow varies between

specific muscle groups, for example the magnitude of blood flow in the gluteus maximus, vastus lateralis, and deltoid muscles differs to such a degree that it affects the rate of drug absorption. These differing rates of absorption also affect drug plasma concentrations after IM administration of drugs, as reported in humans (Evans 1975). Furthermore, at rest, postural muscles, have a larger blood supply when compared to non-postural muscles (Benet et al. 2011). Conversely, the influence of decreased muscle perfusion on absorption rate was demonstrated in humans administered IM injections of the hydrophilic antibiotic, gentamicin, after spinal cord injury. The reported drug absorption rate was lower in paralyzed muscle compared to non-paralyzed muscle probably due to reduced muscle blood flow, whereas volume of distribution (V_d) and clearance (CL) were increased (Segal et al. 1988). Whether injection is IM or intermuscular, defined as injection within the muscle or between the muscle fascias respectively, also affects the rate of drug absorption (Groothuis et al. 1980).

2.5.2 Distribution

After a drug molecule has been absorbed, it has to reach its site of action at a sufficient concentration to elicit a biological response. Distribution is the process by which the drug molecules are transported to tissue by the circulation. Passive transport of a drug is dependent on four main factors: (i) the properties of the drug (most importantly lipid solubility, molecular weight and pK_a as described earlier); (ii) the concentration gradient between blood and tissue; (iii) the ratio of blood flow to tissue mass and, (iv) the affinity of the drug to tissue constituents.

Both importation and exportation of drugs to and from cells by active transporters is adenosine triphosphate (ATP)-dependent, thus requiring energy (Al-Shawi & Senior 1993). Crone et al. (1965) demonstrated carrier-mediated transport of a solute across the BBB for the first time with dextrose. The distribution of drugs is mediated by efflux and influx drug transporters. Additionally, the protein binding of drug molecules influence distribution since only free drug molecules can penetrate tissue membranes.

The body fluids are divided into three major compartments: intravascular, interstitial and intracellular fluid. After IM injection, whether a molecule distributes only into the blood or all three compartments, affects its concentration. A molecule that distributes only into plasma

achieves higher concentrations in plasma than a molecule that distributes into interstitial and intracellular fluid as well. Additionally, the blood flow within the tissue affects the distribution; high blood flow per unit mass of tissue as well as large tissue mass contribute to chemical accumulation of drug molecules into that tissue. Brain, heart, kidney, liver and endocrine glands are tissues with high blood flow. Muscles and skin have intermediate blood flow per tissue mass whereas adipose tissue and bones have low blood flow.

Binding to plasma proteins is one of the factors influencing drug disposition. The most important binding proteins in the serum are albumin and α_1-acid glycoprotein (Bohnert & Gan 2013). When a molecule binds to plasma protein, this newly-bound complex travels through the circulation until it dissociates. Usually this happens when the affinity of the drug for another molecule, such as a transporter protein, or tissue component is greater than that for the plasma protein. The effect of the drug in the body is usually dependent upon the unbound concentration of drug in the plasma. Therefore, changes in the concentration of plasma proteins may change the amount of the unbound drug in the circulation. Additionally, co-administered drugs may lead to competition of the same binding sites in plasma proteins (Israili & Dayton 2001).

2.5.2.1 BBB and blood-spinal cord barrier (BSCB)

The BBB is a protective mechanism that forms a lipophilic, semipermeable interface between the brain parenchyma and the blood stream. It mediates the entry of nutrients, ions and other molecules to brain parenchyma, whilst, protecting the brain from xenobiotics *e.g.* toxins, and pathogens (Daneman 2015).

The BBB is comprised of brain microvascular endothelial cells that form the walls of the capillaries, astrocytes, pericytes and the basement membrane (Figure 2) (Zlokovic 2008, He Y et al. 2014, Yao et al. 2014). Beneath the endothelial and epithelial cells in the brain, lies the basement membrane (Hynes et al. 2009, Baeten et al. 2011, Kim et al. 2011), a 50-100 nm thick protein sheath (Vracko 1970, Ruben 1994) that surrounds the endothelial cells and pericytes (Engelhardt & Sorokin 2009). Basement membrane has many important functions, such as: structural support; signalling transduction and cell anchoring (Hynes et al. 2009, Kim et al. 2011, Baeten et al. 2011). Pericytes are a type of mesenchymal cell that are embedded in the basement membrane and located in the perivascular space, between the

capillary wall and astrocytic end-feet. Pericytes probably play a critical role in the synthesis of the basement membrane components, such as proteoglycans, but their contribution to the system is poorly understood. The basement membrane seems to be maintained by all the cells of the BBB: endothelial cells; pericytes and astrocytes (Engelhardt & Sorokin 2009, Cardoso et al. 2010).

Astrocytes form part of the BBB and they are essential for the development, maintenance and function of this barrier. (Bauer & Bauer 2000, Abbot 2006, Engelhardt & Sorokin 2009, Cardoso et al. 2010). More than contributing to the physical properties of the BBB, astrocytes modulate the BBB via secretory mechanisms that in turn establish the properties of cellular components (Bauer & Bauer 2000, Abbot 2006, Engelhardt & Sorokin 2009, Cardoso et al. 2010).

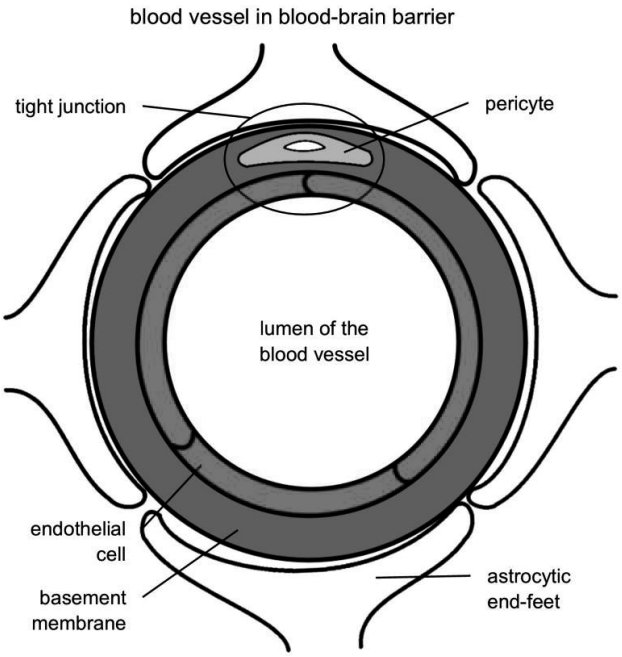

Figure 2. The anatomical structure of the BBB.

The BSCB is a morphologically similar structure to the BBB (Bartanusz et al. 2011). Compared to the BBB, the BSCB is more permeable to, for example cytokines, and regional differences in the permeability of the spinal cord to cytokines have been detected (Pan et

al. 1997). Ge & Pachter (2006) studied cultured microvascular endothelial cells from the murine spinal cord and demonstrated decreased expression of tight junction-associated proteins in spinal cord when compared to brain endothelial cells. However, the expression of some tight junction-associated proteins remained unchanged (Ge & Pachter 2006). These findings may explain the differences in the permeability of BSCB and the BBB to some drugs (Pan et al. 1997).

2.5.2.2 Transport through the BBB

In order to penetrate the BBB and thereby gain access to brain tissue, a molecule has to pass through two membranes of the endothelial cells in the BBB. Three important factors modulating and controlling drug disposition in the CNS are passive membrane permeability, active transport through the BBB and the degree of tissue binding between the brain and plasma compartments (Jeffrey & Summerfield 2010).

Physiological tissue barriers, such as the BBB or BSCB, affect the distribution of drug molecules. If a molecule is unable to pass through these tissue layers on its own, for example owing to its size or low lipid solubility, it needs assistance from active transporter proteins. Transportation through the BBB includes both uptake mechanisms and efflux transporters. In addition to these mechanisms, astrocytes and other cells can release chemical factors modulating the permeability of the endothelial cells very quickly, over seconds to minutes (Abbott, 2002).

Tight junctions between endothelial cells limit the movement of hydrophilic molecules through the BBB, while, small lipophilic substances, such as oxygen and carbon dioxide, diffuse freely according to their partial pressure gradients (Grieb et al. 1985). There is a strong association between the penetration of drugs into BBB and their lipophilicity (Rapoport 1976, Pardridge 1981). For example, the α_2-adrenoceptor agonists used as sedatives are lipid soluble molecules that have a low molecular weight (< 400 Da) and therefore are able to cross the BBB (Salonen 1989).

Protein-transporters are located within the cell membrane and by virtue of their location they effectively mediate the pharmacological characteristics of many clinically used drugs (Fromm, 2000, Ayrton & Morgan, 2001, Borst & Elferink, 2002, Schinkel & Jonker, 2003, Fromm 2004). Peptide receptors of the BBB regulate various processes, such as

transcytosis of a ligand from the blood into the brain or from brain to blood (Pardridge 1986, 2012). There are over 400 membrane transporters that belong to two superfamilies – ATP-binding cassette (ABC) and solute carriers (SLC) (Schinkel & Jonker 2003, Hediger et al. 2004, Giacomini & Sugiyama 2006). The process of ABC-transporters requires ATP hydrolysis for these efflux transporters to be able to export molecules (Dallas et al. 2006). Organic cation transporters (OCTs) and organic anion transporters (OATs) belong to the SLC superfamily (Hediger et al. 2004, Koepsell et al. 2007). They transport hydrophilic, organic substrates with low molecular mass (typically less than 400 Da) (Ahlin et al. 2008).

The dog has 52 genes responsible for encoding ABC proteins (Dean & Annilo 2005). These proteins are grouped into seven families from ABCA to ABCG (Hediger et al. 2004). ABC-transporters, such as the breast cancer resistance protein (BCRP, ABCG2), extrude endogenous and exogenous substrates across membranes; they protect cells from potential toxins by transporting substrate drugs from the cytoplasm out of the cell (Vlaming et al. 2009). P-glycoprotein is an efflux transporter coded by the ABCB1 gene, formerly known as the multi drug resistance gene (MDR1) (Ueda et al. 1987). P-glycoprotein is the best known ABC transporter in veterinary medicine, first reported in *mdr1a(-/-)* knockout mice (Schinkel et al. 1994, 1995). In addition to the BBB and BSCB, P-glycoprotein (P-gp) can be found in epithelial cells in kidney, liver, testicles, placenta, lungs and intestine. In addition, it is expressed in neoplastic cells (Litman et al. 2001, Leslie et al. 2005, Cartwright et al. 2012).

The human breast cancer resistance protein (ABCG2, BCRP) is another of the better known ABC transporters within the BBB (Begley 2004, Daunchy et al. 2008, Kamiie et al. 2008, Miller 2010). It limits the bioavailability of some substrates (van Herwaarden & Schinkel, 2006, Vlaming et al. 2009, Mealey et al. 2011). In addition to previously mentioned transporters, multiple additional transporters take part in the active passage of drugs through the BBB.

2.5.2.3 What affects the permeability of the BBB?

Whereas lipid-soluble molecules diffuse through the BBB (Liu et al. 2004) described before, high polar surface area and the tendency to form hydrogen bonds restrict the drug molecules from accessing the CNS since these characteristics delay the movement of molecules from aqueous phase into the lipid cell membrane (Clark 2003).

Clineschmidt et al. (1988) speculated that the most probable causes for the low concentration of vatinoxan found in the rat brain tissue were its low fat solubility, relatively large molecular size and the barrier functions of the BBB. The octanol-water partition coefficient, defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase in a two-phase octanol/buffer system, describes the behaviour of a molecule in these two phases (Miller et al. 1985). However, the octanol-buffer distribution coefficient is 1.27 for morphine (Plummer et al. 1990) which is very similar to the 1.3 for vatinoxan (Clineschmidt et al. 1988). Therefore, as morphine is known to penetrate CNS, the lipophilicity of vatinoxan is probably not sufficiently low to explain its poor penetration into the CNS.

The molecular weight of a drug affects its permeability through the BBB; the molecular weight of medetomidine is 200 g/mol (Pubchem), whereas that of vatinoxan is 419 g/mol (Pubchem). Although the cut off value for significant molecule passage through the BBB cannot be stated definitively, it is between 400 and 657 g/mol (Levin et al. 1980), suggesting that the size of vatinoxan molecule may reduce its permeability.

Bennett et al. (2017) suggested in an *in vitro* study using human P-gp that vatinoxan was probably not a P-gp substrate within the BBB. However, Bennett et al. (2017) could not rule out the possibility that vatinoxan was a substrate for other efflux transporters which, have not yet been identified. Furthermore, dexmedetomidine showed no evidence of active transport (Bennett et al. 2017). However, the importance of P-gp within the BBB has been reported in dogs administered macrocyclic lactones such as ivermectin and loperamide (Mealey et al. 2001, 2008). A deletion mutation in the gene coding for canine P-gp may lead to increased sensitivity to certain drugs, such as butorphanol, in dogs homozygous for this mutation (Wandel et al. 2002, Sartor et al. 2004, Mealey 2006). This is evident as symptoms of toxicity with avermectin parasiticides such as ivermectin (Mealey et al. 2001). This mutation is seen in for an example herding breeds, such as Collies (Mealey 2001, 2008). However, mutations in other transporters and their clinical relevance have not been studied as widely as P-gp and therefore they are not described fully by the author nor recognised as frequently in the literature.

Many pathological conditions modulate the permeability of the BBB. Many inflammatory mediators increase the diameter of the blood vessels and increase their permeability, inducing leakage across the BBB (Abbot 2000), which can lead to brain oedema (Stamatovic et al. 2008). Several types of molecules, such as histamine, matrix

metalloproteases, cytokines, lipids, free radicals and growth factors can increase permeability (Stamatovic et al. 2008).

In conclusion, the characteristics of the molecule, especially its liposolubility, ionization and molecule size affect its capacity to passively cross the BBB. In addition, the disease state, especially inflammatory or ischaemic states, affect the BBB itself through proinflammatory mediators, thus modulating its permeability. Furthermore, active transportation of a drug molecule through the BBB can be modulated by inhibition or activation of drug transporters.

2.5.3 Metabolism

In drug metabolism, a drug is processed via metabolic pathways, usually in the liver, to modify its chemical structure. Usually the main purpose of metabolism is to ensure elimination by increasing the water solubility of the molecule. Drug metabolism can be divided into three phases. In the first phase metabolic enzymes add polar or reactive groups to the drug molecule. The four pathways available are oxidation, reduction, hydration and hydrolysis. The cytochrome P450 (CYP) superfamily of enzymes have an important role on phase I liver metabolism through oxidation (Shapiro & Shear, 2002). In the second phase drug molecules are conjugated into a polar compound mainly catalysed by transferase enzymes. The conjugated drug molecules may be further modified if needed. Thereafter, the metabolites are usually recognised by efflux transporters and moved out of the cells.

2.5.4 Excretion

Excretion is the process of removal of the drug or its metabolites from the body. The ultimate route for elimination is kidney, but drugs and their metabolites can additionally be excreted in bile, sweat, saliva, tears, milk and expired air. The degree of ionization and water solubility determine how much of the drug is excreted via the renal route. In laboratory beagles, vatinoxan ameliorated dexmedetomidine-induced changes in blood flow of the kidney detected with contrast-enhanced ultrasound (Restitutti et al. 2013).

2.6 Pharmacological drug interactions

When two or more drugs are administered concomitantly, chemical or pharmacological drug-drug interactions may develop. A pharmacological drug interaction is described as a change in the action of a drug due to concomitant administration of another drug, food or supplement (Shapiro & Shear, 2002). The pharmacodynamic drug-drug interactions by receptor agonism and antagonism have been described earlier. The most common pharmacokinetic drug interactions are the inhibition or activation of enzymes taking part in the metabolism of the drugs, or the activation and inhibition of transporter proteins (Shapiro & Shear, 2002).

While the cardiovascular effects of medetomidine are alleviated, vatinoxan also accelerates the IM absorption of medetomidine via a probable improvement in perfusion. Simultaneously, vatinoxan deepens the medetomidine-evoked sedation through increased plasma drug concentrations when given concomitantly using a single injection, as reported in dogs (Restitutti et al. 2017). Similar findings have been later reported also in cats (Honkavaara et al. 2017, Pypendop et al. 2017).

Vatinoxan increased the MAC of sevoflurane in dogs, which was speculated to be related to CNS penetration by the drug (Hector et al. 2017). Previously it was speculated that changes in the distribution of concomitantly given drugs and improved CO due to vatinoxan (Honkavaara et al. 2012) would explain the need for higher doses of co-administered drugs. However, Hector et al. (2017) administered vatinoxan alone, so changes in clearance or distribution of α_2-adrenoceptor agonists did not explain this finding. MAC of volatile anaesthetics is regulated through effects at the spinal cord level (Eger et al. 2002), and there is proof that the permeability of the BSCB and BBB differs to interferons, for example (Pan et al. 1997, Bartanusz et al. 2011). Hence, if vatinoxan penetrates BSCB more easily than BBB, this might explain the higher MAC of volatile anesthetics in the presence of vatinoxan. Therefore, the effects of vatinoxan on the MAC of sevoflurane (Hector et al. 2017) might be also explained by variation in the permeability of BSCB and the BBB (Pan et al. 1997). Changes in cardiovascular performance, for an example through improved CO, might affect the distribution of sevoflurane, which could be one factor explaining the findings reported by Hector et al. (2017). However, atipamezole has been shown to have no effect on the MAC of isoflurane in rats and dogs after the administration of α_2-adrenoceptor agonists (Ewing et al. 1993, Eger et al. 2003).

Inducers or inhibitors of CYP-enzymes can induce or inhibit the metabolism of drugs by influencing their clearance. For example, medetomidine is a CYP2B11 inhibitor (Baratta et al. 2009), and it may inhibit the clearance of some drugs, such as midazolam and ketamine. It has been suggested that this drug-drug interaction is one explanation for the dose-sparing effects of medetomidine (Baratta et al. 2009). Drug interactions are possible at the level of the BBB, because it is not just a passive anatomical barrier, but also a complex active interface expressing a variety of active influx and efflux transporters. The inhibition and activation of transporter proteins is an important drug interaction mechanism; whereas ketoconazole and omeprazole work as inhibitors of P-gp, rifampicin acts as an inducer for P-gp (Solhaug et al. 2019). Molecules can work as both substrates and inhibitors. For a clinically relevant example, methadone works as a substrate for cytochromes CYP2B6 and CYP3A4/5 as well as P-glycoprotein; however, it works as an inhibitor for the enzyme CYP2D6 (Solhaug et al. 2017). In renal cells, clarithromycin decreases secretion of digoxin through the inhibition of P-gp in the kidney cells (Wakasugi et al. 1998).

Drug elimination is also dependent on the perfusion of the organs in question. As medetomidine reduces CO (Thurmon et al. 1994, Sinclair et al. 2003), it may delay or decrease drug excretion of concomitantly given drugs via the renal route. Atipamezole, on the other hand, accelerates the elimination phase of medetomidine; CL increases and T½ decreases (Salonen et al. 1995). These changes are supposed to be dependent on the restoration of haemodynamic function following the atipamezole administration to dogs treated with α_2-adrenoceptor agonists. Vatinoxan also increases early disposition of dexmedetomidine following an IV bolus administration by doubling the apparent V_z and CL of dexmedetomidine (Honkavaara et al. 2012, Bennett et al. 2016). The effect on clearance of α_2-adrenoceptor agonists is probably mediated via improvement or restoration of CO,which enhances hepatic perfusion.

In conclusion, generally important mechanisms for pharmacological drug-drug interactions are the induction or inhibition of drug metabolism through enzymes, inhibition or activation of transporter proteins, pharmacodynamic potentiation and drug antagonism, such as competitive inhibition. However, particularly α_2-adrenoceptor agonists and antagonists may also induce pharmacokinetic drug-drug interactions by altering blood circulation.

3 AIMS OF THE STUDY

1. To describe the effects vatinoxan on plasma concentrations and especially on absorption of medetomidine (MED) and certain other drugs when administered concomitantly intramuscularly in healthy dogs (I, III)
2. To compare the concurrent concentrations of vatinoxan and the enantiomers of medetomidine in canine plasma and CNS after intravenous co-administration (I, II)
3. To confirm the effects of vatinoxan on the clinical characteristics of medetomidine-butorphanol combination in healthy client-owned dogs sedated for routine radiographic imaging. (III)

4 MATERIALS AND METHODS

4.1 Ethical approval

The National Animal Experimental board provided ethical approval for all of the studies (ESAVI/7187/04.10.03/2012, study I), (ESAVI-2010-04178/Ym23, study II) and (ESAVI/6082/04.10.07/2016, study III).

4.2 Animals

The laboratory beagles used in studies I and II were considered healthy based on recent clinical and neurological examinations, complete blood counts and serum chemistry. For study I, six purpose-bred beagle dogs (four castrated males and two ovariohysterectomized females, mean weight ± standard deviation (SD) 14.4 ± 1.6 kg) were used. These beagles have now been rehomed and enjoying their life as pets. In study II, six research Beagle dogs (four females, two males, mean weight 12.5 ± 1.4 kg) scheduled for euthanasia for reasons other than this study (Heikkilä et al. 2018), were used. Beagles used in study I and II were housed according to the European Union guidelines (63/2010/EU) in groups in indoor pens with access to outdoor runs so that they were exposed to both artificial (from 7:00 to 16:00) and natural light. The indoor environmental temperature was between 15 and 24 ºC. Food was withheld for 12 hours before each study, water was freely available.

For the study III, 57 client-owned dogs requiring sedation for non-invasive diagnostic imaging purposes were used. The dogs weighed 5 kg or more and were between 3 months and 10 years of age.

4.3 Instrumentation

In all studies, a 20 or 22-gauge intravenous (IV) catheter (Terumo Europe N.V., Belgium) was placed either in the cephalic vein (I, III) or lateral saphenous vein (II).

In study I, a 16 gauge single lumen central venous catheter (MILA International Inc, KY, USA) was inserted aseptically into a jugular vein for blood collection under local anaesthesia (5 mg lidocaine subcutaneously, Lidocain 20 mg, Orion Pharma, Finland) and fixed to the

adjacent skin with topical tissue adhesive (Gluture, World precision instruments, FL, USA). A 3-way stopcock was attached to this catheter to enable blood collection.

4.4 Treatments, drugs and doses

The study number, treatments and drug doses are listed in Table 2. In study I there was a 14-day wash-out period between treatments. In study I, random allocation was performed drawing lots: three tickets with the word “treatment”, and three tickets with the word “control” were drawn in a randomized order. I study III, seven blocks with 4-10 animals were created to get an even distribution of sizes and breed types between the treatment groups. Randomization was performed within these blocks by using a specific software by a person who did not take part in the study otherwise.

Study	**Number and source of dogs**	**Route**	**Treatment**	**Study design**
I	6 Laboratory beagles	IM	1) medetomidine hydrochloride 20 µg/kg + butorphanol 100 µg/kg + midazolam 200 µg/kg (MBM) 2) MBM + vatinoxan hydrochloride 500 µg/kg (MBM-V)	Prospective, randomized cross-over study
II	6 Laboratory beagles	IV	medetomidine 40 µg/kg + vatinoxan 800 µg/kg	Prospective, experimental trial
III	57 Privately owned dogs	IM	1) medetomidine 0.5 mg/m^2 + butorphanol 0.1 mg/kg (MB) 2) medetomidine 0.5 mg/m^2 + vatinoxan 10 mg/m^2 + butorphanol 0.1 mg/kg (MBV)	Prospective, randomized, clinical trial

In study I, 1 mL of medetomidine solution (1 mg/mL of medetomidine hydrochloride (HCl) (Dorbene 1 mg/mL, Vetcare Oy, Finland) and 1 mL of saline solution (Natriumklorid, B. Braun, 9 mg/mL) were mixed in an ampoule containing 25 mg of vatinoxan hydrochloride powder. For the treatment without vatinoxan, 1 mL of medetomidine hydrochloride solution was mixed with 1 mL of saline. The drugsolutions (MBM or MBM-V) were drawn up into syringes and mixed with butorphanol (Torpudor 10 mg/mL, Richter Pharma AG, Austria) and midazolam (Midazolam Hameln 5 mg/mL, Hameln Pharmaceuticals Gmbh, Germany). The solution was confirmed to be colourless, clear, and transparent. Injection was administered into lumbar epaxial muscles with the dogs in lateral recumbency. The contralateral sites were used for the second treatments.

In study II, vatinoxan was diluted in a similar manner to study I. A dose of 40 µg/kg medetomidine HCl was used (Dorbene 1 mg/mL, Laboratories Syva, , Spain) with 800 µg/kg of vatinoxan HCl (Vetcare Ltd,, Finland) was administrated as a rapid IV bolus. The dogs were euthanized 20 minutes after medetomidine and vatinoxan administration with an overdose of IV pentobarbital (140 mg/kg, Euthasol 400 mg/mL, Le Vet BV, Netherlands).

In study III, dogs were given either treatment MB or MBV. Dosage of medetomidine was calculated based on body surface area using the formula: body surface area = $10.1 * kg^{2/3} * 10^{-2}$ (White & Kearney 2014, Pypendop & Jones 2015). The dose of medetomidine was equivalent to a dose of 29.5 µg/kg for a 5 kg dog and 11.7 µg/kg for an 80 kg dog. A solution of 0.5 mg/mL of medetomidine HCl (vials for experimental use were provided by Vetcare Ltd, Finland) was used for MB-treatment, and for MBV, a solution containing 0.5 mg/mL of medetomidine HCl and 10 mg/mL of vatinoxan was used. Butorphanol was drawn up into a separate syringe but mixed with medetomidine solution in a single syringe before administration IM into the gluteal muscles.

4.5 Assessment of sedation

In studies I and III, the assessment of the level of sedation was performed with both a visual analogue scale (VAS; analog scale of 0-100) and a descriptive sedation score (DSS) (Table 3). VAS and DSS were assessed by the same person, blinded to treatment, before and 3, 6, 10, 15, 20, 25, 30, 40, 50, 60 and 90 minutes after injection in study I. In study III, sedation was assessed at 0, 5, 10 and then at 10 minute intervals after drug administration.

Table 3. Descriptive sedation score
0 = No sedation
1 = Can stand / walk, but ataxic
2 = Recumbent, strong resistance to lateral positioning
4 = Recumbent, no resistance to lateral positioning, does not react to hand clap

4.6 Assessment of injection reaction

Reactions to injection (not to needle insertion), were scored in study III according to Table 4.

Table 4. Injection reaction.
0 = No reaction
1 = Mild reaction, non-specific change in posture, subtle movement without specific attention to the injection site
2 = Moderate reaction, specific change in posture and movement, specific attention to the injection site
3 = Severe reaction, attempt to move away from injection, vocalization, aggression, biting, growling or barking

4.7 Drug analyses

In all of the studies, blood samples were collected into Ethylenediaminetetraacetic acid (EDTA) -tubes (Vacuette, Grainer Bio-One, NC, USA) for drug plasma concentration analyses at 30, 60, 120, 240 and 360 minutes after the drug injection (study I), 20 minutes after drug injection (study II) and within a 20 minute time frame (study III). Plasma samples were separated by centrifugation and frozen at -20 °C until analysed. In study II, after confirmation of euthanasia by auscultating the lack of heartbeat, the CNS was quickly removed. Exsanguination was not performed prior to sample collection. Prior to freezing, the dura mater was opened, and any remaining spinal fluid or blood was absorbed with a gauze from the tissue samples. Tissue samples were obtained from the lumbar and cervical spinal intumescences and from the brain; immediately frozen in liquid nitrogen and stored at -80 °C. All drug analyses were performed with liquid chromatography tandem mass

spectrometry (LC-MS/MS). A summary of the drugs analysed in each study is presented in Table 5.

In study I, the linear range of the assay was 25 ng/mL to 460 ng/mL. For quality control samples, the inter-assay accuracy (at 70, 250 and 380 ng/mL) was 94.4 % - 111.6 %. Validation was performed for range, precision, accuracy, carry-over, interference of analytes, internal standards and analyte stability for both analytical methods. The inter-assay accuracy for quality control samples (at 70, 250 and 380 ng/mL) was 94.4 % - 111.6 %. Validation was performed for range, precision, accuracy, carry-over, interference of analytes, internal standards and analyte stability for both analytical methods. For more details, see article I.

Table 5. Summary of drug concentrations analysed with LC-MS/MS

Study	Drugs analysed	Matrix of analysis
I	Dexmedetomidine, levomedetomidine, butorphanol, midazolam, vatinoxan	Plasma
II	Dexmedetomidine, levomedetomidine, vatinoxan	Plasma, tissue from the CNS
III	Medetomidine, butorphanol, vatinoxan.	Plasma

For the analysis of plasma samples in study II, dexmedetomidine, levomedetomidine and vatinoxan were combined into one working solution. Working solutions were formulated by dilution of stock solutions (1 mg/mL) with methanol to concentrations of 0.01, 0.1, 1, 10 and 100 ng/µL. Calibrators were formulated at concentrations ranging from 0.1 to 2000 ng/mL. For each quantitative assay, fresh calibration curves and negative control samples were produced. For tissue sample analyses in study II, accuracy for QC samples was 89 - 107 % and imprecision was 3 - 6 % for all analytes. The technique was optimised to provide a quantitation limit of 0.5 ng/mL for dexmedetomidine and levomedetomidine and 0.1 ng/mL for vatinoxan. The detection limit was approximately 0.2 ng/mL for dexmedetomidine and levomedetomidine, and for vatinoxan it was 0.05 ng/mL.

In study III, reference standards were prepared in dog plasma spiking with the analytes making a final concentration range of 0.02–20 000 ng/mL. The linear calibration ranges (ng/mL) were undiluted butorphanol 0.2–500, diluted medetomidine 0.2–2000, and undiluted

vatinoxan 0.5–2000. The QC samples were in the range 94-116% of the nominal concentrations. For details, see Study III.

Dexmedetomidine concentrations were analysed after solid phase extraction with Sep-Pak tC18 96 well extraction plates (Waters Co, MA, USA) and 4,5-diphenylimidazole (Sigma-Aldrich, , MO, USA) was used as an internal standard.

Tissues samples (230-890 mg) were weighed into 7 mL Precellys hard tissue homogenizing vials (Bertin Corp,, MD, USA) and then placed into a freezer at -20 ºC for 10 minutes (study II). After this, samples were homogenized for 30 seconds at 5500 rpm in a Precellys 24 tissue homogenizer (Bertin Corp., USA), followed by a 5-minute cool down period. Then samples were homogenized again at 15000 g for 2 minutes with 1 mL of CAN containing d4OHD and HEPS at 100 ng/mL. After this, 0.5 mL was transferred to a 13 X 100 mm glass tube and dried in nitrogen at 45 ºC.

Samples were redissolved with 100 µL of 5% ammonium hydroxide and 500 µL of water in a saturated sodium chloride solution before adding 3 mL of methyl tert-butyl ether (MTBE). Samples were mixed by rotation for 20 minutes at 40 revolutions per minute. Following rotation, samples were centrifuged 4 ºC for 5 minutes at 2260 g. The top organic layer was moved to a glass tube. Again, samples were dried in nitrogen at 45 ºC and dissolved in 200 µL of 20 mM ammonium formate with 0.1% diethylamine (DEA). The injection volume for LC-MS/MS system was 10 µL. Positive heated electrospray ionization at 300 ºC was used. TSQ Vantage triple quadrupole mass spectrometry (Thermo Scientific, CA, USA) was used for quantitative analysis of tissue, coupled with a turbulent flow chromatography system (TFC TLX2 Thermo Scientific, CA, USA) having LC-10ADvp LC systems (Shimadzu, Japan) that was operated in laminar flow mode. For a detailed description, see study II. Calibration curves and quantities of dexmedetomidine, levomedetomidine and vatinoxan in all samples were generated with Quanbrowser software (Thermo Fisher Scientific, USA). For all calibration curves, a weighting factor of 1/X was used. Accuracy for QC samples was 89 - 107 % and imprecision 3 - 6 % for all analytes. The technique was optimized to provide a quantitation limit of 0.5 ng/mL for dexmedetomidine and levomedetomidine and 0.1 ng/mL for vatinoxan. The detection limit was around 0.2 ng/mL for dexmedetomidine and levomedetomidine, and for vatinoxan it was 0.05 ng/mL.

4.8 Statistical analyses

In study I, a power calculation (http://hedwig.mgh.harvard.edu/sample_size/size.html) was derived from earlier results for T_{max} of dexmedetomidine (Restitutti et al. 2017), butorphanol (Pfeffer et al. 1980), and midazolam (Schwartz et al. 2013) after IM administration in dogs. To detect a 50% decrease in $T_{max,}$ with a power of 80% and an alpha-level set at 0.05, with pairwise one-tailed one-way analysis of variance (ANOVA), three dogs were needed for midazolam, five for dexmedetomidine and six for butorphanol. For study II, the number of dogs was determined by the initial study (Heikkilä et al. 2018), so it could not be altered, and therefore the power analysis was not performed. For study III, power analysis was performed based on a mean HR of 40 bpm (MB) and 60 bpm. Standard deviation was set up at 15 bpm, and with a power of 80% and an alpha-level set up at 0.05, 20 dogs were required for each treatment.

In the study I and III, analyses were computed using SPSS Statistics version 24.0 for Windows (IBM). In study II, statistical analyses were performed with JMP Pro 12 (SAS Institute, NC, USA).

For evaluation of the normality of data distribution, the Shapiro-Wilks test was used for studies I and III. In study II, non-parametric tests were used. Non-parametric tests were chosen for study II because the sample size was small, Irrespective of data distribution, non-parametric tests are more conservative for the analysis of results.

Repeated-measures ANOVA was used for analysis of HR (study I and III) and respiratory rates (III), and t-test followed by the Holm Bonferroni *post hoc* correction was used for comparisons between treatments and against baseline.

Area under the time-concentration curves (AUC) were calculated with the trapezoidal method for sedation during certain time frames ($AUC_{sed0\text{-}15}$) for the first 15 minutes in study I, and AUC_{VAS30} and AUC_{SDS30} for the first 30 minutes in study III). The Mann-Whitney U-test was used for the comparison of AUC_{VAS30} (study III), VAS scores and pale mucous membranes (III) and sedation scores (I) between treatments at each time point. Related Samples Friedman's Two-Way of Analysis of Variance by Ranks with Holm Bonferroni correction was used for testing VAS and pale mucous membranes against baseline within each treatment (study III). Two-sided Fischer's exact test was used to compare the need for additional medetomidine or atipamezole, presence of loose faeces, tiredness (residual

sedation) and decreased appetite. Independent samples T-test was used for plasma drug concentrations and time for “head down” (study III).

Paired samples 1-tailed t-test was used for C_{max} and T_{max}, and 2-tailed t-testing was performed on $AUC_{0\text{-}90}$ and $AUC_{sed0\text{-}15}$ (study I). Friedman’s test was used to compare calculated tissue:plasma ratios between vatinoxan, levomedetomidine and dexmedetomidine, followed by a one-tailed Wilcoxon’s Rank Sum test with a Bonferroni *post hoc* correction. Two-tailed Wilcoxon’s Rank Sum test was used to compare plasma and tissue concentrations between dex- and levomedetomidine (study II).

The alpha level was set to 0.05 in all studies. Data are presented as mean and standard deviations or medians and range.

5 RESULTS

5.1 Sedation

In study I, the VAS sedation measurements are shown in Table 6. Peak sedation scores shown as median (range) was observed at 15 minutes for MBM-V and at 20 minutes for MBM (p = 0.109). $AUC_{sed0\text{-}15}$ was significantly greater for MBM-V than MBM (p = 0.047).

Table 6. Visual analogue scale (VAS) for sedation score (0 - 100) for treatments MBM and MBM-V.
Data of VAS scores are reported as median (range). $AUC_{sed0\text{-}15}$ (reported as mean ± standard deviation (SD)) were calculated for the first 15 minutes. Five dogs were given both treatments (study I).

Time point (minutes)	MBM	MBM-V
Before	0 (0 - 0)	0 (0 – 0)
3	18 (0 – 20)	23 (3 – 85)
6	14 (0 – 51)	84 (6 – 100)
10	69 (22 – 97)	96 (72 – 100) †
15	95 (17 – 100)	100 (96 – 100) †
20	100 (58 – 100) †	100 (100 – 100) †
25	100 (76 – 100) †	100 (100 – 100) †
30	100 (79 – 100) †	100 (83 – 100) †
40	96 (78 – 100) †	94 (87 – 100) †
50	86 (77 – 100) †	78 (66 – 100)
60	85 (70 – 100)	70 (50 – 78)
90	68 (62 – 81)	26 (15 – 74)
$AUC_{sed0\text{-}15}$	598 ± 256 *	996 ± 261 *

***Significant difference between treatments**
† Significant difference compared to baseline.

In study II, all dogs became profoundly sedated after the treatment and spontaneously remained in lateral recumbency until euthanized. In the study III, AUC_{VAS} was significantly

higher for the first 30 minutes in animals that were given vatinoxan (MBV) ($p < 0.001$). Data for VAS, AUC_{VAS} and AUC_{SDS} are presented in Table 7. VAS for MBV was significantly higher compared to baseline from 5 ($p = 0.025$) until 40 minutes ($p < 0.001$). VAS for MB, as well, was higher compared to baseline at 10-40 minutes ($p < 0.001$). More dogs in group MBV required additional medetomidine after 30 minutes ($p = 0.023$) and fewer dogs needed atipamezole ($p < 0.001$) than with MB.

Table 7. The visual analogue sedation scores and area under the curve for visual analogue scale (VAS) sedation score-time (AUC_{VAS30}) and simple descriptive score (AUC_{SDS30}) for the first 30 minutes and. Data are reported as median (range).

Time point (minutes)	MB	MBV
0	0 (0 – 0) n = 29	0 (0 – 0) n = 27
5	58 (9 – 86) n = 28	64 † (21 – 100) n = 27
10	96 † (31 – 100) n = 27	100 † (51 – 100) n = 27
20	100 † (82 – 100) n = 28	100 † (78 – 100) n = 27
30	100 † (83 – 100) n = 28	100 † (71 – 100) n = 27
40	100 † (90 – 100) n = 26	100 † (14 – 100) n = 25
AUC_{VAS30}	2445 * (1855 – 2585)	2555 * (1868 – 2750)
AUC_{SDS30}	98 (75 – 105) †	105 (73 – 110) †

*** Significant difference between treatments**

† Significant difference from baseline

5.2 HR

In study I, HR was significantly higher after MBM-V than MBM between 20 and 90 minutes (Figure 3, upper picture). In study III, HRs were significantly lower with both treatments

compared to baseline. HR was significantly higher between 10-40 minutes with MBV than MB. HRs are reported until 40 minutes (Figure 3, lower picture): at that time point data from two dogs were missing from both groups [procedure had already ended (n = 1 in both groups) and one dog had needed additional sedation (n = 1 in both groups)]. The lowest HRs detected after additional medetomidine was 28 beats per minute (bpm) with MB (n = 1) and 34 bpm with MBV (n = 7). The ranges in HRs after additional medetomidine were between 28 - 36 bpm in MB and 34 - 68 bpm in MBV.

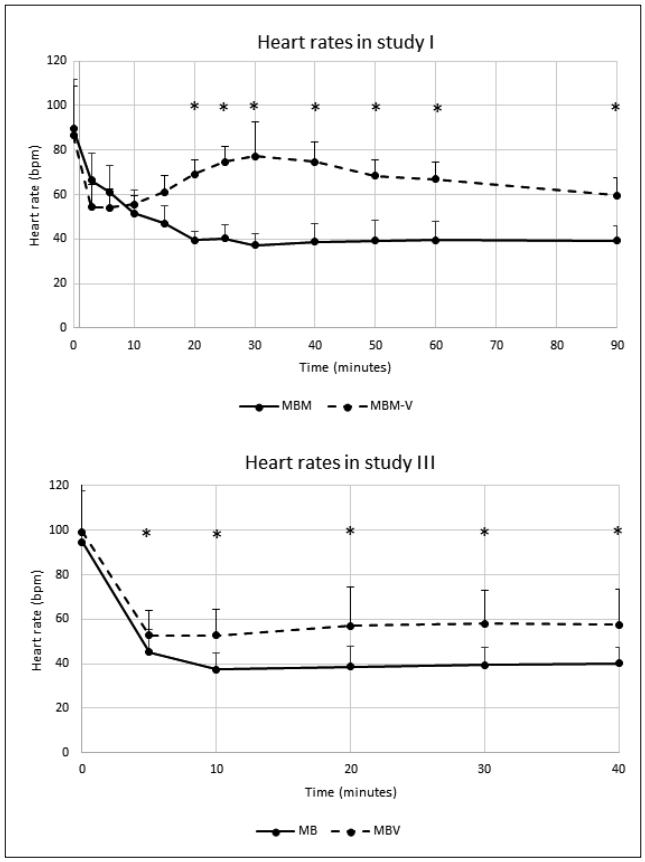

Figure 3. Heart rate after IM administration of MBM and MBM-V drug combinations (study I, upper picture). In study III, heart rates were reported until 40 minutes after injection of MB or MBV for dogs that were not given additional medetomidine or atipamezole (lower picture). * Significant difference between treatments.

5.3 Concentrations of drugs in plasma and tissue

Plasma concentrations of levomedetomidine, dexmedetomidine, butorphanol, midazolam and vatinoxan are presented in Figures 4 - 8. Table 8 summarizes C_{max}, T_{max} and area under curve for the first 90 minutes (AUC_{0-90}) for these drugs. T_{max} of midazolam and levomedetomidine were significantly earlier after MBM-V. The other analytes showed a tendency towards higher C_{max} and smaller T_{max} after MBM-V compared to MBM.

Table 8. Observed peak drug concentrations in plasma (C_{max}), the time of maximum drug concentration in plasma (T_{max}) and area under the concentration-time curve (AUC). Shown as mean ± SD and minimum and maximum in paretheses.

*** Significant difference between treatments**

Drug	Treatment	C_{max} (ng/mL)	T_{max} (minutes)	AUC_{0-90} (minutes * ng/mL)
Dexmedetomidine	MBM	4.3 ± 2.0 (0.9 – 6.0)	27 ± 15 (10 – 50)	216 ± 92 (54 – 279)
	MBM-V	6.6 ± 2.6 (3.7 – 10.8)	17 ± 4.5 (10 – 20)	247 ± 65 (144 – 307)
	p-value	0.09	0.10	0.63
Levomedetomidine	MBM	2.7 ± 1.3 (0.5 – 3.7)	32 ± 15 (10 – 50)	140 ± 63 (30 – 181)
	MBM-V	4.6 ± 1.6 (2.6 – 6.7)	18 ± 6 (10 – 25)	178 ± 47 (107 – 227)
	p-value	0.08	0.036 *	0.38
Butorphanol	MBM	10.7 ± 6.1 (1.7 – 16.9)	27 ± 4.5 (10 – 30)	589 ± 305 (116 - 886)
	MBM-V	19.9 ± 9.6 (10.5 – 34.0)	15 ± 5 (10 – 20)	818 ± 246 (535 – 1143)
	p-value	0.07	0.07	0.33
Midazolam	MBM	82.2 ± 43.9 (12.9 – 134.0)	23 ± 9 (10 – 40)	3743 ± 1886 (749 – 5837)
	MBM-V	157.8 ± 95.8 (82.9 – 300.2)	11 ± 6 (6 – 20)	5644 ± 2213 (3920 – 8900)
	p-value	0.11	0.049 *	0.33
Vatinoxan	MBM-V	907 ± 173 (672 – 1051)	23 ± 6 (15 – 30)	62755 ± 11268 (49563 – 77548)

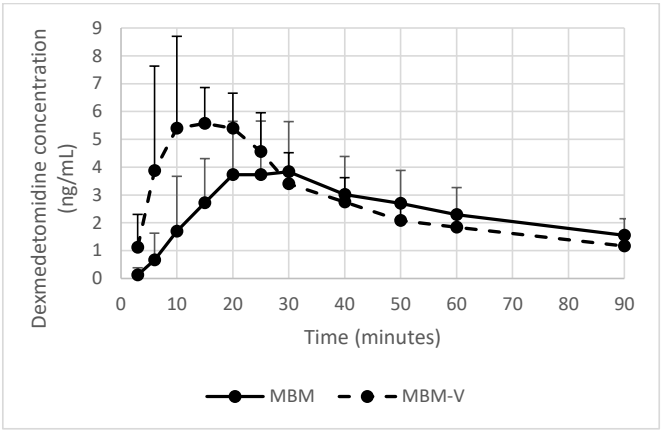

Figure 4. Dexmedetomidine concentrations in plasma after administration of 1) medetomidine hydrochloride (20 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM), and; 2) medetomidine (20 µg/kg) + vatinoxan hydrochloride (500 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM-V). Data from five dogs are shown. Both treatments were administered at 0 minutes. Data are shown as mean and standard deviation (SD).

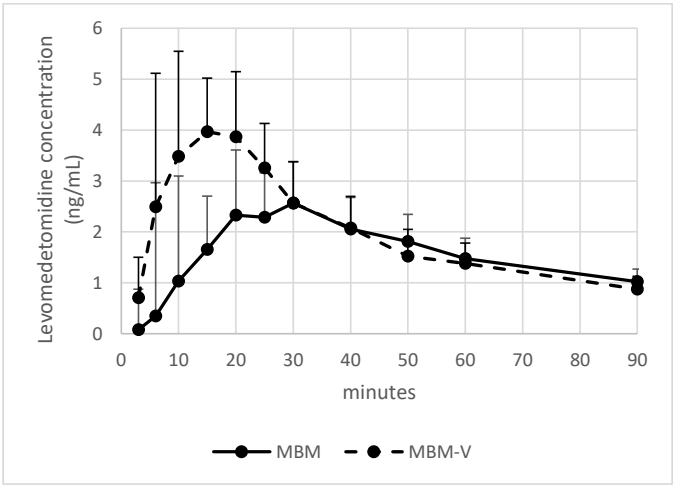

Figure 5. Levomedetomidine concentrations in plasma after administration of 1) medetomidine hydrochloride (20 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM), and; 2) medetomidine (20 µg/kg) + vatinoxan hydrochloride (500 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM-V). Data from five dogs are shown. Both treatments were administered at 0 minutes. Data are shown as mean and standard deviation (SD).

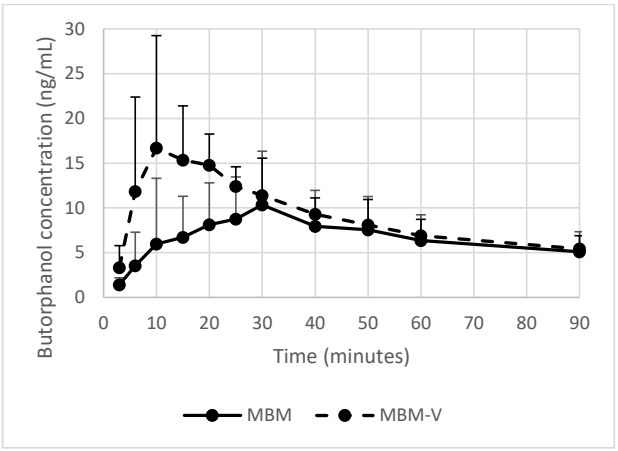

Figure 6. Butorphanol concentrations in plasma after administration of 1) medetomidine hydrochloride (20 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM), and; 2) medetomidine (20 µg/kg) + vatinoxan hydrochloride (500 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM-V). Data from five dogs are shown. Both treatments were administered at 0 minutes. Data are shown as mean and standard deviation (SD).

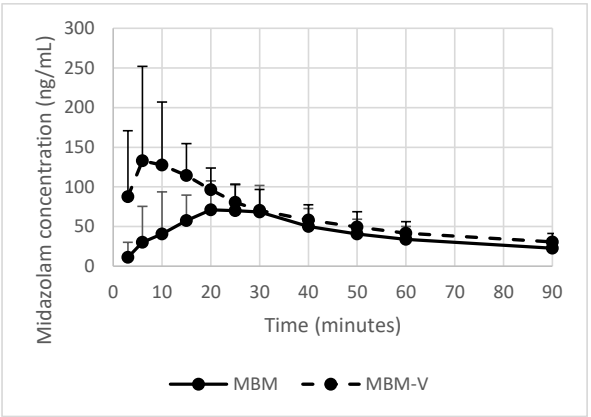

Figure 7. Midazolam concentrations in plasma after administration of 1) medetomidine hydrochloride (20 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM), and; 2) medetomidine (20 µg/kg) + vatinoxan hydrochloride (500 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM-V). Data from five dogs are shown. Both treatments were administered at 0 minutes. Data are shown as mean and standard deviation (SD).

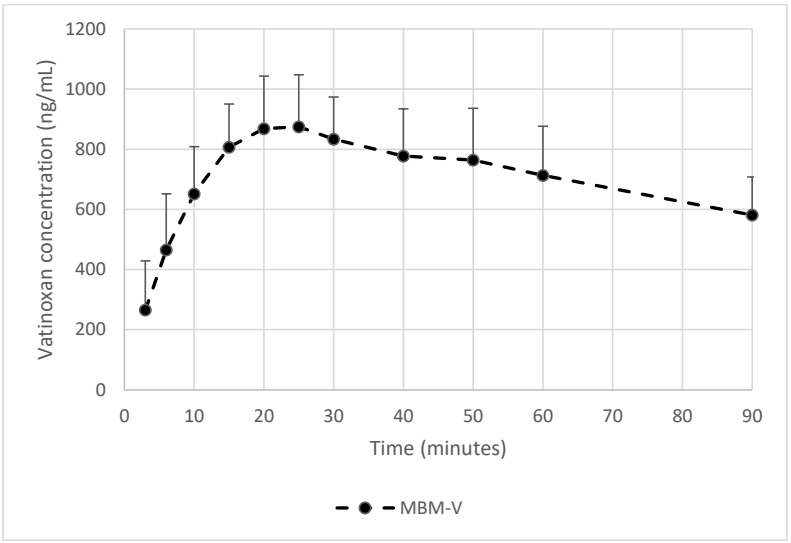

Figure 8. Vatinoxan concentrations in plasma after administration of 1) medetomidine hydrochloride (20 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM), and; 2) medetomidine (20 µg/kg) + vatinoxan hydrochloride (500 µg/kg) + butorphanol (100 µg/kg) + midazolam (200 µg/kg) IM (MBM-V). Data from five dogs are shown. Both treatments were administered at 0 minutes. Data are shown as mean and standard deviation (SD).

Data from one of the six dogs had to be excluded from study I because low concentrations of vatinoxan (9.59 - 36.5 ng/mL) were found in its blood samples also after MBM treatment. The reason for this contamination with vatinoxan could not be detected.

For study II, the concentrations for dexmedetomidine, levomedetomidine and vatinoxan and calculated ratios between plasma *versus* brain and cervical and lumbar spinal cord are summarized in Table 9. No macroscopic findings indicative of subclinical disease were present within the CNS or other examined organs in the dogs.

Table 9. Concentrations of dexmedetomidine (DEX), levomedetomidine (LEVO), vatinoxan (VAT) and VAT/DEX ratio [shown as median (minimum and maximum range)] in plasma, brain, and cervical (c) and lumbar (l) spinal cord. The concentration ratios of each tissue to plasma are also presented.

	DEX	LEVO	VAT	Ratio VAT/DEX
Plasma (ng/mL)	6.8 (5.9 - 7.8)	4.1 (3.4 - 5.2)	1380 (701 -2440)	202 (115 - 323)
Brain (ng/g)	29.9 (27.1 - 39.7)	28.2 (22 - 37.3)	27.5 (15.6 - 44.3)	0.8 (0.65-1.53)
Spinal cord (c) (ng/g)	23.3 (17.8 - 31.2)	18.5 (15.4 - 27.8)	17.2 (12.7 - 20.6)	0.7 (0.6 - 0.88)
Spinal cord (l) (ng/g)	22 (14.5 - 31.3)	17.8 (12.9 - 31)	20 (12.4 - 28.3)	0.9 (0.73 - 1.2)
Ratio plasma/brain	0.2 (0.16 - 0.26)	0.1 (0.14 - 0.18)	52.2 (28.4 - 67.2)	
Ratio plasma/spinal cord (c)	0.3 (0.19 - 0.33)	0.2 (0.18 - 0.28)	81.4 (78.5 - 192.1)	
Ratio plasma/spinal cord (l)	0.3 (0.22 - 0.36)	0.2 (0.17 - 0.31)	63.3 (56.5 - 97.2)	

In study III, plasma concentrations of both medetomidine and butorphanol (Figures 9 and 10) were significantly higher with MBV ($p < 0.001$) in samples collected between 11 and 18 minutes after drug injection. Vatinoxan plasma concentration in MBV-treated dogs was 607 ± 204 ng/mL.

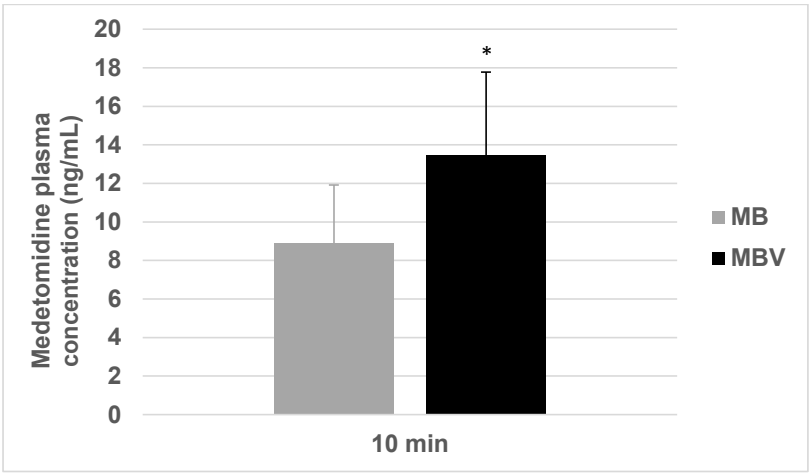

Figure 9. Plasma concentrations of medetomidine in 56 pet dogs undergoing sedation with either MB (n = 29) or MBV (n = 27) for radiography. Plasma concentrations are reported as mean ± standard deviation (SD). * Significant difference between treatments.

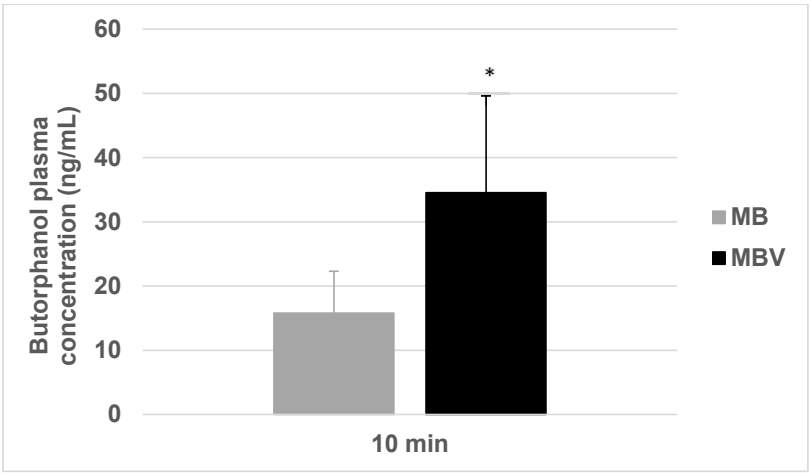

Figure 10. Plasma concentrations of butorphanol in 56 pet dogs undergoing sedation with either MB (n = 29) or MBV (n = 27) for radiography. Plasma concentrations are reported as mean ± SD * Significant difference between treatments.

Other results from study III are presented in Table 10. “Head down” time, mucous membrane colour, requirement for additional medetomidine or atipamezole, duration of required sedation, tiredness (residual sedation), loose faeces, decrease in appetite, number of blood samples analysed and sampling time are presented in Table 10. No significant differences were detected between treatments in injection reactions (p = 0.117) (Study III).

Table 10. Results for 56 client-owned dogs undergoing sedation with either MB (n = 29) or MBV (n = 27) for radiography.

	MB	**MBV**	**p-value**
Head down (seconds) (mean ± standard deviation, SD)	298 ± 74	241 ± 78	0.012
Additional medetomidine (number of dogs) (administration time in minutes, mean ± standard deviation, SD)	1 (30)	7 (53 ± 10)	0.023
Atipamezole administration (number of dogs)	26	12	< 0.001
Duration of required sedation (minutes, mean ± standard deviation, SD)	66 ± 14.5	66 ± 19	
Lethargy (number of dogs)	26	17	0.221
Loose faeces (number of dogs)	7	9	0.558
Decrease in appetite (number of dogs)	2	3	0.664
Blood sample analysed (number of dogs)	23	25	
Blood sampling time (minutes, range)	13 (11 – 15)	13.5 (12 – 18)	0.36

Tiredness, loose faeces and decrease in appetite reported by owners.
One dog in each group was given both additional medetomidine and atipamezole.

6 DISCUSSION

6.1 Intramuscular absorption

Studies I and III were designed to investigate how the addition of vatinoxan to a combination of sedative drugs would affect the IM absorption of the concomitantly administered drugs. The IM route was chosen for its practicality and its clinical relevance. The main finding was that the addition of vatinoxan to the sedative combination increased the absorption of both medetomidine and other concomitantly given drugs, such as butorphanol (I, III) and midazolam (I) after IM administration. This is clinically relevant as it may suggest a need for adjustment of drug doses when they are used concomitantly with vatinoxan. The results from study I suggest that the pharmacological drug interactions induced by α_2-adrenoceptor agonists and their antagonists began during the drug absorption phase from the extravascular site of administration. The alleviation of α_2-adrenoceptor agonist-induced vasoconstriction probably leads to improved perfusion within the muscle, and therefore increases drug uptake into the circulation. It has been speculated that the improved absorption seen with the administration of vatinoxan is caused by inhibition of the local vasoconstrictive effect medetomidine at the site of the injection (Restitutti et al. 2017).

Butorphanol (I, III) and midazolam (I) were used as marker molecules to describe the effects of medetomidine and vatinoxan on IM absorption of concomitantly given drugs. These two drugs were chosen for their common use in veterinary medicine in combination with α_2-adrenergic agonists. Both of the drugs are short-acting and both are rapidly absorbed following IM injection. Butorphanol is a synthetic κ-agonist-μ-antagonist opioid (Raffe 1989, Hosgood 1990, Commiskey et al. 2005) that is often combined with α_2-adrenoceptor agonists to improve the quality of sedation in dogs (Ko et al. 1996, Pypendop et al. 1996, Kuo & Keegan 2004). Midazolam, is a benzodiazepine which induces muscle relaxation and tranquillisation in dogs (Gross et al. 1990), and therefore it is often combined with α_2-adrenergic agonist for minor procedures (Verstegen & Petcho 1993, Pypendop et al. 1996, Itamoto et al. 2000). These marker molecules revealed that α_2-adrenoceptor agonists and antagonists may also affect the absorption of other simultaneously administered drugs (study I).

In linear pharmacokinetics, the T1/2 elimination half-life of a drug molecule is constant, and not dependent on the absorption phase. However, this is not always true. Flip-flop

pharmacokinetics describe a situation, where following extravascular dosing the rate of absorption of a drug is slower than its elimination, as reported here with medetomidine when it is administered IM without vatinoxan (Study I, Restitutti et al. 2017). Since the elimination cannot take place before the drug is in systemic circulation, the decrease in the terminal slope of the concentration curve depends greatly on how quickly the drug is absorbed (Yáñez et al. 2011). Since vatinoxan accelerates the absorption of concomitantly given drugs after IM injection, it also seems to affect the elimination rate of medetomidine (study I, Restitutti et al. 2017) more so than reported following IV injection (Honkavaara et al. 2012, Bennett et al. 2016).

6.2 Sedation and other central effects of vatinoxan

The distribution of vatinoxan to both brain and spinal cord was poor in dogs, whereas enantiomers of medetomidine concentrated in the CNS (study II). The brain:plasma ratio for vatinoxan was 0.02 (0.01-0.04) in dogs (study II), which was comparable to results reported for marmosets (0.04) and rats (0.06) (Clineschmidt et al. 1988). Salonen et al. (1989) also reported that medetomidine concentration in rat brain was significantly higher compared to plasma 20 minutes after subcutaneous administration. Concentrations of medetomidine and vatinoxan in spinal cord have not been reported previously in dogs or other species. The results of study II confirm the peripheral distribution of vatinoxan in dogs. This has already been proposed earlier since vatinoxan alleviates the peripheral effects of α_2-adrenoceptor agonists without affecting their centrally mediated effects in dogs (Enouri et al. 2008, Honkavaara et al. 2011, Restitutti et al. 2017).

In studies I and III, vatinoxan intensified the early-stage sedation and shortened its duration in dogs. In all studies, reliable clinical sedation was achieved both in the presence (study I, II and III) and in the absence (studies I and II) of vatinoxan. The changes in the depth of sedation, when vatinoxan was added to the IM administered sedative combination, may be divided into two phases. Firstly, the onset of sedation was faster (study III). The overall sedative effect was also significantly greater during the first 15 (study I) or 30 (study III) minutes when vatinoxan was administered IM in the same syringe with other drugs. Secondly, the presence of vatinoxan shortened the duration of sedation (studies I and III). Similar findings were reported by Restitutti et al. (2017), who also detected that the time-concentration curves of dexmedetomidine in plasma intersected approximately 30 minutes

after IM administration of medetomidine and medetomidine-vatinoxan. Prior to the 30-minute time point the plasma concentration of dexmedetomidine (stereoisomer dexmedetomidine was analysed although medetomidine was administered) was higher in the presence of vatinoxan, whereas after this time point, the concentrations of dexmedetomidine in plasma were higher in dogs given medetomidine alone (Restitutti et al. 2017). The drug concentrations in plasma detected in studies I and III agreed with these earlier results (Restitutti et al. 2017).

The impact of vatinoxan on α_2-adrenoceptor agonist-induced sedation appears to be minimal and related to its effects on the disposition of co-administered drugs via the IM route in dogs (Restitutti et al. 2017), cats (Pypendop et al. 2017, Honkavaara et al. 2017), and sheep (Adam et al. 2018). Since the addition of vatinoxan to the sedative combination of medetomidine-butorphanol or medetomidine-butorphanol-midazolam shortens the duration of the sedation, it may be beneficial to consider additional sedative strategies in animals requiring longer sedation. However, in the presence of vatinoxan, a deep level of sedation is usually achieved during the initial 20-30 minutes. The sedation develops between 5 and 10 minutes after IM injection (studies I and III). This time frame is usually sufficient for minor procedures, such as routine diagnostic imaging.

Bennett et al. (2017) speculated that active transport out of the cell might play some role in the efflux of vatinoxan through the BBB, in which transporters other than P-gp could be involved. If an efflux transporter were involved in this process, a high concentration of vatinoxan could occur within the CNS. This in turn might prevent or reverse the sedation induced by α_2-adrenoceptor agonists in animals with a mutation in the gene encoding that specific protein. In study III, it would have been interesting to analyse DNA-samples from the individual dogs since many breeds, such as for an example Border Collies, were represented, some of which are known to carry the ABCB1-gene defect (Mealey et al. 2001, Neff et al. 2004, Mealey et al. 2008). If any of these individuals had been homo- or heterozygous for the mutation in the ABCB1-gene the length and depth of sedation or recovery in dogs may have been apparent. Although, multiple individuals from many herding breeds participated in study III, but no excessive enhanced sedation effects were seen.

6.3 Bradycardia

Medetomidine-induced bradycardia was apparentafter IM administration of medetomidine-vatinoxan in studies I and III. After the absorption phase, vatinoxan attenuated the bradycardia, but in the first 5-10 minutes the decrease in HR caused by medetomidine was evident after both treatments (studies I and III). The initial decrease in HR in dogs given vatinoxan may have been caused by the more rapid absorption of medetomidine in the presence of vatinoxan (Restitutti et al. 2017). Restitutti et al. (2017) also reported the initial bradycardia after medetomidine, but the classical cardiovascular changes, such as reduction in HR, CO and increase in central venous pressure, were attenuated by vatinoxan.

In the first study, the initial bradycardia was evident probably due to the higher plasma concentrations of dexmedetomidine in the presence of vatinoxan. The T_{max} of dexmedetomidine occurred at approximately 15 minutes, whereas the T_{max} of vatinoxan was approximately 25 minutes (Study I, Restitutti et al. 2017). Therefore, the early concentration of vatinoxan may not have been sufficiently high to compete with dexmedetomidine at peripheral α_2-adrenoceptors and prevent the initial bradycardia seen in study I. Hence, when dexmedetomidine is absorbed more rapidly in the presence of vatinoxan, the α_2-adrenoceptor-induced bradycardia may be even more prominent than that reported in the absence of vatinoxan. If vatinoxan were given prior to the administration of an α_2-adrenoceptor agonist, it could reach peripheral vascular adrenoceptors before the agonist and alleviate even the initial vasoconstriction and baroreflex-mediated bradycardia. In dogs given vatinoxan as a pretreatment 30 minutes before administration of 5 µg/kg of dexmedetomidine, vatinoxan prevented the immediate pressor effects of dexmedetomidine and no significant difference in HR was detected at 5 and 60 minutes after dexmedetomidine (Pagel et al. 1998). Additionally, when these drugs were given at the same time, but injected into different muscles, the concurrent IM administration of vatinoxan and medetomidine performed at least as well as IV route for attenuation of cardiovascular changes caused by medetomidine (Rolfe et al. 2012).

Vatinoxan alleviated medetomidine-induced bradycardia in healthy dogs, but even with vatinoxan present, HRs were lower than baseline. With medetomidine, HRs were approximately 40 bpm whereas in medetomidine-vatinoxan-treated dogs, they were approximately 60 bpm. Commonly 50 to 60 bpm is regarded as the threshold for bradycardia in large dogs, and 70 bpm in small breed dogs (Thurmon et al. 2007, Brodbelt et al. 2008).

In studies I and III, HRs were often below these thresholds, even with vatinoxan. However, Hall et al. (1991) reported very low resting HRs in adult clienbt-owned dogs of various breeds. During natural sleep minimum HRs ranging between 17 and 46 bpm were detected with ambulatory electrocardiography. Cruz et al. (2017) also reported mean minimum heart rates below 50 bpm in healthy adult dogs. In beagles, nightly HRs between 60 and 70 bpm have been reported previously (Nolan et al. 2004, Honkavaara et al. 2008). Results from the previous studies performed in dogs during their physiological sleep may indicate that healthy, adult dogs are able to tolerate HRs below the recognised thresholds indicative of bradycardia.

In laboratory beagles, arterial and central venous pressures were significantly lower and closer to the physiological status following the administration of medetomidine-butorphanol-vatinoxan IM compared to medetomidine-butorphanol-treatment (Salla et al. 2014b). Salla et al. (2014b) did not detect hypotension in dogs given medetomidine-butorphanol-vatinoxan following IM or IV administration. Meanwhile after medetomidine-butorphanol-treatment dogs had significantly higher MAPs for the first 30 minutes after IM injection. Furthermore, vatinoxan alleviated the cardiovascular effects of medetomidine specifically changes in HR, cardiac index and oxygen delivery index were significantly higher after both IM and IV administration routes in dogs given vatinoxan (Salla et al. 2014b). Since only HRs were reported in studies I and III, without data on blood pressure or any other cardiovascular variables, aside from the colour of the mucous membranes in study III, it cannot be proven that the alleviation of the medetomidine-induced bradycardia actually improved cardiac function. However, it may be speculated that the alleviation of vasoconstriction could have resulted in hypotension in studies I and III. This may have overridden the beneficial effects of a higher HR in vatinoxan-treated dogs compared to dogs given medetomidine without vatinoxan. However, the findings reported by Salla et al (2014b) indicate that the detected increase in HR (I, III) in vatinoxan-medicated dogs could mirror with improvement in cardiac function.

6.4 Adverse effects of vatinoxan

In study III, the most frequently observed side-effect after sedation was loose faeces. All dogs expressing loose faeces were administered either atipamezole, vatinoxan or both. The adrenergic regulation of gastrointestinal motility and secretions is mainly related to α_2-adrenoceptors (Virtanen & McDonald, 1987). Prolonged intestinal transit time (Ruwart et al. 1980) and inhibition of gastric acid secretion in rats (Del Tacca et al. 1982) and dogs (Soldani et al. 1984) induced by α_2-adrenoceptor agonists have been demonstrated. These effects were inhibited by α_2-adrenoceptor antagonists such as atipamezole (Virtanen & McDonald, 1987). Atipamezole restores intestinal motility and induces defaecation in dogs after α_2-adrenoceptor agonist-induced sedation (Maugeri et al. 1994) and vatinoxan seems to have a similar effect (study III). When both of these drugs (atipamezole and vatinoxan) are given, it may further enhance gastrointestinal motility. Hence, it would be beneficial to inform the owners about this possible adverse effect after administration of α_2-adrenoceptor antagonists (study III).

In studies I and III, dogs were studied at ambient room temperatures of and they maintained normal body temperature without active warming and there were no significant differences between treatments. However, as reported by Vainionpää et al. (2013), the alleviation of vasoconstriction by vatinoxan may lead to increased core heat loss in dogs handled at similar room temperatures to those used in studies I and III. Therefore, in clinical work, it is essential to monitor the body temperature in sedated and anaesthetized dogs, since vatinoxan-treated dogs may be prone to hypothermia (Vainionpää et al. 2013).

6.5 Methodological considerations and limitations of the studies

In studies I and III, power analyses were performed, and the number of dogs included in were expected to provide sufficient statistical power to avoid type II errors. Unfortunately, in study I, one of the six beagles had to be excluded due to technical failure. Therefore, a larger sample size was required in study I, to increase statistical power. Had the sample size been higher, more the differences in T_{max} and C_{max} of dexmedetomidine and butorphanol may have been statistically significant as well. In study I, at the time of administration, the temperature of the drug combination was not measured. Differences in the temperature of

the two solutions may have affected the absorption rate from the site of injection. Although, with both treatments the drugs were handled in the same manner, and held in a hand warm water bath, so it is unlikely that the outcome would have differed between these two treatments.

There were some limitations in study II. After euthanasia, exsanguination was not performed. Therefore, contamination of tissue samples with blood may have resulted in higher concentrations of vatinoxan, thus narrowing the difference between the plasma and CNS drug concentrations. Hence, The plasma:brain ratio of vatinoxan in study II may have been greater than reported here However, the very low concentration of vatinoxan in the CNS did not confirm lack of pharmacological action *per se*. It is remains unclear what CNS concentration of vatinoxan fails to produce a clinically relevant competition with dexmedetomidine at central adrenoreceptors. Further studies are required to determine the role of active transporters in the influx and efflux of vatinoxan to and from the CNS. Additionally, it would have been beneficial to determine the plasma and CNS concentrations of the individual drugs when administered as a sole agent.

There was only one time point reported for drug plasma concentrations in studies II and III. Blood samples taken at multiple time points would have provided additional information about absorption and distribution, but the protocol of the studies limited the possibility for this (scheduled euthanasia in study II *versus* client-owned dogs in study III).

In study III, it would have been useful to measure arterial blood pressures, even if non-invasively, to verify the alleviation of the α_2-adrenoceptor agonist-induced vasoconstriction by vatinoxan. This would also have indicated if hypotension occurred in dogs given a drug combination including vatinoxan. We did not demonstrate any improvement in CO. Since the alleviation of medetomidine induced vasoconstriction by vatinoxan may have resulted in hypotension, which could have overridden the beneficial effects of the higher HRs in dogs treated with the medetomidine-butorphanol-vatinoxan combination.

The studies presented special ethical considerations. Trials were planned on the basis that the laboratory beagles used in study I would be rehomed without any permanent or lasting physiological effects. When designing the studies, the focus was to limit the stress experienced by the dogs, and thereby to minimize the associated elevation in their endogenous catecholamine concentrations. Dogs in study II were euthanized for other purposes and the protocol was applied solely to facilitate their terminal anaesthesia (Heikkilä

et al. 2018). Dogs in study III were pet dogs admitted for routine diagnostic imaging; no surgical or otherwise painful procedures, aside from injecting the sedative combination IM and obtaining IV access for blood sample collection a, were performed.

6.6 Clinical implications and future prospects

Study III was the first published report where pet dogs of various breeds were treated with medetomidine and vatinoxan. Based on those results, vatinoxan proved to be clinically usable for healthy dogs as a part of a sedative combination for minor procedures since it alleviates medetomidine-induced bradycardia. No significant differences were detected in the number and type of side-effects that occurred in dogs given vatinoxan (MBV) compared to dogs administered MB. With respect to clinical use, the shorter onset of sedation and higher peak concentrations of drugs in plasma should be noted. This is beneficial as the preparation for the procedure can be started sooner. On the contrary, the shortened duration of sedation needs to be considered when planning the protocol for the sedation.

Since the alleviation of the undesirable cardiovascular effects of α_2-adrenoceptor agonists can be achieved with the use of vatinoxan (studies I, III), it may widen the spectrum of dogs and clinical conditions, for which α_2-adrenoceptor agonists can be used. Further studies are required in dogs that are not deemed clinically healthy, since vatinoxan has not been studied in dogs that have systemic disease. Additionally, further studies investigating the use of vatinoxan as a part of an anaesthetic protocol for surgical procedures are required, as these findings have not yet been reported. Specifically, the effect of vatinoxan on antinociceptive effects of coadministered drugs during surgery would be warranted for its clinical use.

7 CONCLUSIONS

1. Vatinoxan increased early plasma concentrations of concomitantly IM administered drugs by accelerating the absorption, probably by reversing α_2-agonist-evoked vasoconstriction. This resulted in an increase in the early plasma concentrations of medicines (I, III).
2. Low brain concentration and high plasma concentration of vatinoxan confirmed its peripheral disposition. On the contrary, the enantiomers of medetomidine, dex- and levomedetomidine, had higher concentrations in brain than in plasma. This finding explains why vatinoxan alleviated peripheral adverse effects of α_2-adrenoceptor agonists without affecting their CNS effects in dogs (II).
3. Vatinoxan intensified the early-stage sedation and shortened its duration as well as alleviating medetomidine-induced bradycardia in healthy client-owned dogs (I, III).

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REFERENCES

Abbott NJ (2002) Astrocyte-endothelial interactions and blood-brain barrier permeability. *Journal of Anatomy*, 200(6), 629-638. doi:10.1046/j.1469-7580.2002.00064.x

Adam M, Raekallio MR, Keskitalo T et al. (2018) The impact of MK-467 on plasma drug concentrations, sedation and cardiopulmonary changes in sheep treated with intramuscular medetomidine and atipamezole for reversal. *Journal of Veterinary Pharmacology and Therapeutics*, 41(3), 447-456. doi:10.1111/jvp.12486

Ahlin G, Karlsson J, Pedersen JM et al. (2008) Structural requirements for drug inhibition of the liver specific human organic cation transport protein 1. *Journal of Medicinal Chemistry*, 51(19), 5932. doi:10.1021/jm8003152

Ahlquist RP (1948) A study of the adrenotropic receptors. *The American Journal of Physiology*, 153(3), 586. doi:10.1152/ajplegacy.1948.153.3.586

Al-Shawi MK & Senior AE (1993) Characterization of the adenosine triphosphatase activity of chinese hamster P-glycoprotein. *The Journal of Biological Chemistry*, 268(6), 4197.

Altman JD, Trendelenburg AU, MacMillan L et al. (1999) Abnormal regulation of the symphathetic nervous system in α_{2A}-adrenergic receptor knockout mice. *Molecular Pharmacology* 56, 154-161.

Ayrton A & Morgan P (2001) Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica; the Fate of Foreign Compounds in Biological Systems*, 31(8-9), 469-497. doi:10.1080/00498250110060969

Baeten KM & Akassoglou K (2011) Extracellular matrix and matrix receptors in blood-brain barrier formation and stroke. *Developmental Neurobiology*, 71(11), 1018-1039. doi:10.1002/dneu.20954

Baratta MT, Zaya MJ, White JA et al. (2009) Canine CYP2B11 metabolizes and is inhibited by anesthetic agents often co-administered in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, 33, 50–55.

Bartanusz V, Jezova D, Alajajian B et al. (2011). The blood-spinal cord barrier: Morphology and clinical implications. *Annals of Neurology*, 70(2), 194-206. doi:10.1002/ana.22421

Bauer HC & Bauer H (2000) Neural induction of the blood-brain barrier: Still an enigma. *Cellular and Molecular Neurobiology*, 20(1), 13-28.

Baxter JS & Evans JM (1973) Intramuscular injection in the cat. *The Journal of Small Animal Practice*, 14(5), 297-302.

Becker DE & Reed KL (2012) Local anesthetics: Review of pharmacological considerations. *Anesthesia Progress*, 59(2), 90. doi:10.2344/0003-3006-59.2.90

Ben-Abraham R, Ogorek D, Weinbroum AA (2000) Dexmedetomidine: A promising agent for anesthesia and perioperative care. *Israel Medical Association Journal*, 2(10), 793-796.

Benet LZ, Kroetz DL, Sheiner LB (2011) Pharmacokinetics: The dynamics of drug absorption, distribution and elimination. *In Goodman and Gilman's the pharmacological basis of therapeutics* (12th edition). New York: McGraw-Hill Education LLC.

Bennett MR, Cheung A, Brain KL (1998) Sympathetic neuromuscular transmission at a varicosity in a syncytium. *Microscopy Research and Technique*, 42(6), 433-450.

Bennett RC, Salla KM, Raekallio MR et al. (2016). Effects of MK-467 on the antinociceptive and sedative actions and pharmacokinetics of medetomidine in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, 39(4), 336-343. doi:10.1111/jvp.12292

Bennett R, Palviainen M, Peltoniemi M et al. (2017) The role of active transport in the transcellular movement of the peripheral α 2 -adrenoceptor antagonist, MK-467: An in vitro pilot study. *Canadian Journal of Veterinary Research*, 81(4), 318-320.

Bloor BC, Frankland M, Alper G et al. (1992) Hemodynamic and sedative effects of dexmedetomidine in dog. *Journal of Pharmacology and Experimental Therapeutics*, 263(2), 690-697.

Bohnert T & Gan LS (2013) Plasma protein binding: From discovery to development. *Journal of Pharmaceutical Sciences*, 102(9), 2953-2994. doi:10.1002/jps.23614

Borst P & Elferink RO (2002) Mammalian ABC transporters in health and disease (ATP-binding cassette). *Annual Review of Biochemistry*, 71, 537.

Brede M, Nagy G, Philipp M et al. (2003). Differential control of adrenal and sympathetic catecholamine release by alpha 2-adrenoceptor subtypes. *Molecular Endocrinology*, 17(8), 1640. doi:10.1210/me.2003-0035

Bylund DB (1988) Sub types of α 2-adrenoceptors: Pharmacological and molecular biological evidence converg. *Trends in Pharmacological Sciences*, 9(10), 356-361. doi:10.1016/0165-6147(88)90254-4

Bylund D, Eikenberg DC, Hieble JP et al. (1994). International union of pharmacology nomenclature of adrenoceptors. *Pharmacological Reviews*, 46(2), 121-136.

Cardoso FLF, Brites D, Brito MA (2010) Looking at the blood-brain barrier: Molecular anatomy and possible investigation approaches. *Brain Research Reviews*, 64(2), 328. doi:10.1016/j.brainresrev.2010.05.003

Cartwright TA, Campos CR, Cannon RE (2012) MillerMrp1 is essential for sphingolipid signaling to p-glycoprotein in mouse blood–brain and blood–spinal cord barriers. Journal of Cerebral Blood Flow & Metabolism, 33(3), 381-388.

Chiba S & Yang SP (2003) Neuroeffector mechanisms involved in the regulation of dog splenic arterial tone. *Journal of Pharmacological Sciences*, 92(2), 84-92 doi:10.1254/jphs.92.84

Chotani MA, Mitra S, Su BY et al. (2004) Regulation of alpha(2)-adrenoceptors in human vascular smooth muscle cells. American Journal of Physiology. *Heart and Circulatory Physiology*, 286(1), H59. doi:10.1152/ajpheart.00268.2003

Clark DE (2003) In silico prediction of blood–brain barrier permeation. *Drug Discovery Today*, 8(20), 927-933. doi:10.1016/S1359-6446(03)02827-7

Clarke KW, Hall LW (1969) "Xylazine" - a new sedative for horses and cattle. Veterinary Record, 8;85(19), 512-517. doi: 10.1136/vr.85.19.512.

Clarke KW & England GC (1989) Medetomidine, a new sedative-analgesic for use in the dog and its reversal with atipamezole. *Journal of Small Animal Practice*, 30(6), 343-348. doi:10.1111/j.1748-5827.1989.tb01575.x

Clineschmidt BV, Pettibone DJ, Lotti VJ et al. (1988) A peripherally acting alpha-2 adrenoceptor antagonist: L-659,066. *Journal of Pharmacology and Experimental Therapeutics*, 245(1), 32-40.

Clough DP & Hatton R (1981) Hypotensive and sedative effects of alpha-adrenoceptor agonists: Relationship to alpha 1- and alpha 2-adrenoceptor potency. *British Journal of Pharmacology*, 73(3), 595-604.

Clutton RE, Bracken J, Ritchie M (1998) Effect of muscle injection site and drug temperature on pre-anaesthetic sedation in pigs. *Veterinary Record*, 142(26), 718. doi:10.1136/vr.142.26.718

Commiskey S, Fan LW, Ho IK et al. (2005) Butorphanol: Effects of a prototypical agonist-antagonist analgesic on κ-opioid receptors. *Journal of Pharmacological Sciences*, 98(2), 109-116. doi:10.1254/jphs.CRJ05001X

Cruz A, Alfonso A, Oba E, et al. (2017) Scaling Relationships Among Heart Rate, Electrocardiography Parameters, and Body Weight. *Topics in Companion Animal Medicine* 32(2), 66-71.

Daneman R & Prat A (2015) The blood-brain barrier. *Cold Spring Harbor Perspectives in Biology*, 7(1) doi:10.1101/cshperspect.a020412

Dean M & Annilo T (2005) Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annual Review of Genomics and Human Genetics*, (6), 123-142. doi:10.1146/annurev.genom.6.080604.162122

Del Tacca M, Soldani G, Bernardini C et al. (1982) Pharmacological studies on the mechanisms underlying the inhibitory and excitatory effects of clonidine on gastric acid secretion. *European journal of Pharmacology*, 81, 255-261.

Docherty J (2010) Subtypes of functional α_1-adrenoceptors. *Cellular and Molecular Life Sciences* 67, 405-417.

Doze A, Chen B, Maze M (1989) Dexmedetomidine produces a hypnotic-anesthetic action in rats via activation of central alpha-2 adrenoceptors. *Anesthesiology,* 71(1), 75-79.

Dum J, Bläsig J, Herz A (1981) Buprenorphine: Demonstration of physical dependence liability. *European Journal of Pharmacology*, 70(3), 293-300. doi:10.1016/0014-2999(81)90163-1

Dundee JW, Gamble JA, Assaf RA (1974) Plasma-diazepam levels following intramuscular injection by nurses and doctors. *The Lancet*, 304(7894), 1461. doi:10.1016/S0140-6736(74)90130-5

Engelhardt B & Sorokin L (2009) The blood-brain and the blood-cerebrospinal fluid barriers: Function and dysfunction. *Seminars in Immunopathology*, 31(4), 497-511. doi:10.1007/s00281-009-0177-0

Enouri SS, Kerr CL, McDonell WN et al. (2008) Effects of a peripheral alpha2 adrenergic-receptor antagonist on the hemodynamic changes induced by medetomidine administration in conscious dogs. *American Journal of Veterinary Research*, 69(6), 728-736. doi:10.2460/ajvr.69.6.728

Evans EF, Proctor JD, Fratkin MJ et al. (1975) Blood flow in muscle groups and drug absorption. *Clinical Pharmacology & Therapeutics*, 17(1), 44-47. doi:10.1002/cpt197517144

Ewing KK, Mohammed HO, Scarlett JM et al. (1993). Reduction of isoflurane anesthetic requirement by medetomidine and its restoration by atipamezole in dogs. *American Journal of Veterinary Research*, 54(2), 294. Retrieved from https://helka.finna.fi/PrimoRecord/pci.medline8094277

Fagerholm V, Grönroos T, Marjamäki P et al. (2004) Altered glucose homeostasis in α_{2A}-adrenoceptor knockout mice. *European Journal of Pharmacology* 505, 243-252.

Fairbanks CA, Stone LS, Kitto KF et al. (2002) α_{2C}-adrenergic receptors mediate spinal analgesia and adrenergic-opioid synergy. The Journal of Pharmacology and Experimental Therapeutics 300, 282-290.

Flacke WE, Flacke JW, Bloor BC, McIntee DF, and Sagan M (1993) Effects of dexmedetomidine on systemic and coronary hemodynamics in the anesthetized dog. Journal of Cardiothoracic and Vascular Anesthesia, 7:41–49.

Flavahan NA & Vanhoutte PM (1986) Effect of cooling on alpha-1 and alpha-2 adrenergic responses in canine saphenous and femoral veins. *The Journal of Pharmacology and Experimental Therapeutics*, 238(1), 139.

Gaddum J (1937) The quantitative effects of antagonistic drugs. *Journal of Physiology*, 89(89), 7-9.

Ge S & Pachter JS (2006) Isolation and culture of microvascular endothelial cells from murine spinal cord. *Journal of Neuroimmunology*, 177(1-2), 209-214. doi:10.1016/j.jneuroim.2006.05.012

Giacomini K & Sugiyama Y (2006) *Goodman and Gilman's the pharmacological basis of therapeutics*. New York: McGraw&Hill. doi:10.1001/jama.1996.03540120077042

Giovannitti JA Jr, Thoms SM, Crawford JJ (2015) Alpha-2 adrenergic receptor agonists: a review of current clinical applications. *Anesthesia Progress*, 62(1):31-39. doi:10.2344/0003-3006-62.1.31

Granholm M, McKusick BC, Westerholm FC et al. (2007) Evaluation of the clinical efficacy and safety of intramuscular and intravenous doses of dexmedetomidine and medetomidine in dogs and their reversal with atipamezole. *Veterinary Record*, 160(26), 891-897.

Grieb P, Forster RE, Strome D et al. (1985) O2 exchange between blood and brain tissues studied with 18O2 indicator-dilution technique. *Journal of Applied Physiology*, 58(6), 1929-1941. doi:10.1152/jappl.1985.58.6.1929

Grimm KA, Tranquilli WJ, Thurmon JC et al. (2000) Duration of nonresponse to noxious stimulation after intramuscular administration of butorphanol, medetomidine, or a butorphanol-medetomidine combination during isoflurane administration in dogs. *American Journal of Veterinary Research*, 61(1), 42-47.

Groothuis DG, Werdler ME, van Miert AS et al. (1980) Factors affecting the absorption of ampicillin administered intramuscularly in dwarf goats. *Research in Veterinary Science*, 29(1), 116. doi:10.1016/S0034-5288(18)32699-7

Gross ME, Tranquilli WJ, Thurmon JC et al. (1990) Hemodynamic effects of intravenous midazolam-xylazine-butorphanol in dogs. *Veterinary Surgery*, 19(2), 173-180. doi:10.1111/j.1532-950X.1990.tb01163.x

Guimarães S (1987) Alpha 2 -adrenoceptor-mediated responses to so-called selective alpha 1 -adrenoceptor agonists after partial blockade of alpha 1 -adrenoceptors. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 335(4), 397-402. doi:10.1007/BF00165554

Guimarães S & Moura D (2001) Vascular adrenoceptors: An update. *Pharmacological Reviews*, 53(3), 451.

Hall LW, Dunn JK, Delaney M et al. (1991) Ambulatory electrocardiography in dogs. *The Veterinary Record*, 129, 213-216.

Hector RC, Rezende ML, Mama KR et al. (2017) Effects of constant rate infusions of dexmedetomidine or MK-467 on the minimum alveolar concentration of sevoflurane in dogs. *Veterinary Anaesthesia and Analgesia*, 44(4), 755-765. doi:10.1016/j.vaa.2016.12.058

Hediger M, Romero MF, Peng JB et al. (2004) The ABCs of solute carriers: Physiological, pathological and therapeutic implications of human membrane transport proteins. *Pflügers Archiv*, 447(5), 465-468. doi:10.1007/s00424-003-1192-y

Heikkilä HM, Jokinen TS, Syrjä P et al. (2018) Assessing adverse effects of intra-articular botulinum toxin A in healthy beagle dogs: A placebo-controlled, blinded, randomized trial. *PLoS ONE*, 13(1) doi:10.1371/journal.pone.0191043

Honkavaara JM, Raekallio M, Kuusela EK et al. (2008) The effects of L-659,066, a peripheral a2-adrenoceptor antagonist, on dexmedetomidine sedation and bradycardia in

dogs. *Veterinary Anesthesia and Analgesia* 35(5), 409–413. doi:10.1111/j.1467-2995.2008.00403.x

Honkavaara JM, Restitutti F, Raekallio MR et al. (2011) The effects of increasing doses of MK-467, a peripheral alpha2-adrenergic receptor antagonist, on the cardiopulmonary effects of intravenous dexmedetomidine in conscious dogs. *Journal of Veterinary Pharmacology and Therapeutics*, 34(4), 332-337. doi:10.1111/j.1365-2885.2010.01242.x

Honkavaara JM, Restitutti F, Raekallio MR et al. (2012) Influence of MK-467, a peripherally acting a 2-adrenoceptor antagonist on the disposition of intravenous dexmedetomidine in dogs. *Drug Metabolism and Disposition*, 40(3), 445-449. doi:10.1124/dmd.111.042671

Honkavaara J, Pypendop B, Ilkiw J (2017) The impact of MK-467 on sedation, heart rate and arterial blood pressure after intramuscular coadministration with dexmedetomidine in conscious cats. *Veterinary Anaesthesia and Analgesia*, doi://doi.org/10.1016/j.vaa.2016.08.011

Horn PT, Kohli JD, Listinsky JJ et al. (1982) Regional variation in the alpha-adrenergic receptors in the canine resistance vessels. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 318(3), 166-172. doi:10.1007/BF00500476

Hosgood G (1990) Pharmacologic features of butorphanol in dogs and cats. *Journal of the American Veterinary Medical Association*, 196(1), 135-136.

Hunter JC, Fontana DJ, Hedley LR et al. (1997) Assessment of the role of α2-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice. *British Journal of Pharmacology*, 122(7), 1339-1344. doi:10.1038/sj.bjp.0701520

Hynes R (2009). The extracellular matrix: Not just pretty fibrils. *Science*, 326(5957), 1216-1219. doi:10.1126/science.1176009

Israili ZH & Dayton PG (2001) Human alpha-1-glycoprotein and its interactions with drugs. *Drug Metabolism Reviews,* 33(2), 161-235. doi:10.1081/DMR-100104402

Jeffrey P & Summerfield S (2010) Assessment of the blood-brain barrier in CNS drug discovery. *Neurobiology of Disease*, 37(1), 33. doi:10.1016/j.nbd.2009.07.033

Khan ZP, Ferguson CN, Jones RM (1999) Alpha-2 and imidazoline receptor agonists, their pharmacology and therapeutic role. *Anaesthesia*, 54(2), 146-165. doi:10.1046/j.1365-2044.1999.00659.x

Ko JC, Bailey JE, Pablo LS et al. (1996) Comparison of sedative and cardiorespiratory effects of medetomidine and medetomidine-butorphanol combination in dogs. *American Journal of Veterinary Research*, 57(4), 535-540.

Koepsell H, Lips K, Volk C (2007) Polyspecific organic cation transporters: Structure, function, physiological roles, and biopharmaceutical implications. *Pharmaceutical Research*, 24(7), 1227-1251. doi:10.1007/s11095-007-9254-z

Koshimizu TA, Tanoue A, Hirasawa A et al. (2002) Recent progress in alpha1-adrenoceptor pharmacology. *Biological and Pharmaceutical Bulletin*, 25(4), 401-408. doi:10.1248/bpb.25.401

Kuo W & Keegan RD (2004). Comparative cardiovascular, analgesic, and sedative effects of medetomidine, medetomidine-hydromorphone, and medetomidine-butorphanol in dogs. *American Journal of Veterinary Research*, 65(7), 931. doi:10.2460/ajvr.2004.65.931

Kuusela E, Raekallio M, Anttila M et al. (2000). Clinical effects and pharmacokinetics of medetomidine and its enantiomers in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, 23(1), 15-20. doi:10.1046/j.1365-2885.2000.00245.x

Kuusela E, Vainio O, Kaistinen A et al. (2001) Sedative, analgesic, and cardiovascular effects of levomedetomidine alone and in combination with dexmedetomidine in dogs. *American Journal of Veterinary Research*, 62(4), 616-621.

Langley JN (1901). On the stimulation and paralysis of nerve-cells and of nerve-endings: Part I. *The Journal of Physiology*, 27(3), 224. doi:10.1113/jphysiol.1901.sp000868

Langley JN (1905). On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari. *The Journal of Physiology*, 33(4-5), 374. doi:10.1113/jphysiol.1905.sp001128

Langer SZ (1974) Presynaptic regulation of catecholamine release. *Biochemical Pharmacology*, 23(13), 1793-1800. doi:10.1016/0006-2952(74)90187-7

Langer SZ, Shepperson NB, Massingham R (1981) Preferential noradrenergic innervation of alpha-adrenergic receptors in vascular smooth muscle. *Hypertension*, 3, 112-118. doi:10.1161/01.HYP.3.3_Pt_2.I112

Leech CJ & Faber JE (1996) Different alpha-adrenoceptor subtypes mediate constriction of arterioles and venules. *American Journal of Physiology-Heart and Circulatory Physiology*, 270(2), H710-H722. doi:10.1152/ajpheart.1996.270.2.H710

Leslie E, Deeley R, Cole S (2005) Multidrug resistance proteins: Role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicology and Applied Pharmacology*, 204(3). doi:10.1016/j.taap.2004.10.012

Levin VA (1980) Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. *Journal of Medicinal Chemistry*, 23(6), 682. doi:10.1021/jm00180a022

Link RE, Desdai K, Hein L et al. (1996) Cardiovascular regulation in mice lacking α_2-receptor subtypes b and c. *Science* 273, 803-805.

Litman T, Druley TE, Stein WD et al. (2001) From MDR to MXR: New understanding of multidrug resistance systems, their properties and clinical significance. *Cellular and Molecular Life Sciences*, 58(7), 931-959. doi:10.1007/PL00000912

Long KM & Kirby R (2008) An update on cardiovascular adrenergic receptor physiology and potential pharmacological applications in veterinary critical care. *Journal of Veterinary Emergency and Critical Care*, 18(1), 2-25. doi:10.1111/j.1476-4431.2007.00266.x

Ludders JW, Reitan JA, Martucci R et al. (1983) Blood pressure response to phenylephrine infusion in halothane-anesthetized dogs given acetylpromazine maleate. *American Journal of Veterinary Research*, 44(6):996-999.

Lähdesmäki J, Sallinen J MacDonald et al. (2003) Alpha2-adrenergic drug effects on brain monoamines, locomotion, and body temperature are largely abolished in mice lacking the alpha2A-adrenoceptor subtype. *Neuropharmacology*, 44(7), 882-892.

Macdonald E, Scheinin M, Scheinin H et al. (1991) Comparison of the behavioral and neurochemical effects of the 2 optical enantiomers of medetomidine, a selective alpha-2-adrenoceptor agonist. *Journal of Pharmacology and Experimental Therapeutics*, 259(2), 848-854.

MacMillan LB, Hein L, Smith MS et al. (1996) Central hypotensive effects of α_{2A}-adrenergic receptor subtype. *Science* 273, 801-803.

Makaritsis KP, Johns C, Gavras I et al. (1999) Sympato Inhibitory function of the α_{2A}-adrenergic receptor subtype. *Hypertension* 34, 403-407.

Matsui H, Lefkowitz RJ, Caron MG et al. (1989) Localization of the fourth membrane spanning domain as a ligand binding site in the human platelet alpha 2-adrenergic receptor. *Biochemistry*, 28(9), 4125.

Maugeri S, Ferre JP, Intorre L et al. (1994) Effects of medetomidine on intestinal and colonic motility in the dog. *Journal of Veterinary Pharmacology and Therapeutics*, 17(2), 148-154. doi:10.1111/j.1365-2885.1994.tb00225.x

Maze M, Fujinaga A (2000) Analgesic action of nitrous oxide is transduced by alpha- 2 B adrenergic receptors and enkephalin release in rodents. *International Journal of Anaesthesia*, 84(2), 284-285.

Mealey KL, Bentjen S, Gay J et al. (2001) Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. *Pharmacogenetics*, 11(8), 727. doi:10.1097/00008571-200111000-00012

Mealey KL (2006). Adverse drug reactions in herding-breed dogs: The role of P-glycoprotein. *Compendium on Continuing Education for the Practicing Veterinarian*, 28(8), 552.

Mealey KL & Meurs K (2008) Breed distribution of the ABCB1-1Δ (multidrug sensitivity) polymorphism among dogs undergoing ABCB1 genotyping. *Journal of the American Veterinary Medical Association*, 233(6), 921-924. doi:10.2460/javma.233.6.921

Mealey KL (2011). ABCG2 transporter: Therapeutic and physiologic implications in veterinary species. *Journal of Veterinary Pharmacology and Therapeutics*, 35(2), 105-112. doi:10.1111/j.1365-2885.2011.01313.x

Millan M, Lejeune F, Gobert A et al. (2000) S18616, a highly potent spiroimidazoline agonist at alpha(2)-adrenoceptors: II. Influence on monoaminergic transmission, motor function, and anxiety in comparison with dexmedetomidine and clonidine. Journal of Pharmacology and Experimental Therapeutics, 295(3), 1206-1222.

Miller DS (2010) Regulation of P-glycoprotein and other ABC drug transporters at the blood–brain barrier. *Trends in Pharmacological Sciences*, 31(6), 246-254. doi:10.1016/j.tips.2010.03.003

Miller MM, Wasik SP, Lan G et al. (1985) Relationships between octanol-water partition coefficient and aqueous solubility. *Environmental Science & Technology*, 19(6), 522-529

Monteiro ER, Teixeira Neto FJ, Castro VB et al. (2007) Effects of acepromazine on the cardiovascular actions of dopamine in anesthetized dogs. *Veterinary Anaesthesia and Analgesia*, 34(5), 312-321. doi:10.1111/j.1467-2995.2006.00328.x

Neff MW, Robertson KR, Wong AK, et al. (2004) Breed distribution and history of canine mdr1-1Delta, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage. *Proceedings of the National Academy of Sciences of the United States of America*, 2004, 101, 11725–11730.

Nolan ER, Girand M, Bailie M et al. (2004) Short communication. Circadian changes in the QT variability index in the beagle dog. *Clinical and Experimental Pharmacology and Physiology* 31, 783–785.

Pagel PS, Proctor LT, Devcic A et al. (1998) A novel alpha2-adrenoceptor antagonist attenuates the early, but preserves the late cardiovascular effects of intravenous dexmedetomidine in conscious dogs. *Journal of Cardiothoracic and Vascular Anesthesia*, 12(4), 429-434. doi:10.1016/S1053-0770(98)90197-5

Pan W, Banks WA, Kastin AJ (1997) Permeability of the blood–brain and blood–spinal cord barriers to interferons. *Journal of Neuroimmunology*, 76(1-2), 105-111. doi:10.1016/S0165-5728(97)00034-9

Pang C (2001) Autonomic control of the venous system in health and disease - effects of drugs. *Pharmacology & Therapeutics*, 90(2-3), 179-230. doi:10.1016/S0163-7258(01)00138-3

Pardridge WM (1986). Receptor-mediated peptide transport through the blood-brain barrier. *Endocrine Reviews,* 7(3), 314. doi:10.1210/edrv-7-3-314

Pardridge WM (1981). Transport of protein-bound hormones into tissues in vivo. *Endocrine Reviews*, 2(1), 103-123. doi:10.1210/edrv-2-1-103

Pardridge WM (2012). Drug transport across the Blood–Brain barrier. *Journal of Cerebral Blood Flow & Metabolism*, 32(11), 1959-1972. doi:10.1038/jcbfm.2012.126

Pascoe PJ, Raekallio M, Kuusela E et al. (2006) Changes in the minimum alveolar concentration of isoflurane and some cardiopulmonary measurements during three

continuous infusion rates of dexmedetomidine in dogs. Veterinary Anaesthesia and Analgesia, 33:97–103. doi: 10.1111/j.1467-2995.2005.00236.x

Peltonen JM, Pihlavisto M, Scheinin M (1998). Subtype-specific stimulation of [35S]GTPgammaS binding by recombinant alpha2-adrenoceptors. European Journal of Pharmacology, 21;355(2-3): 275-279. doi: 10.1016/s0014-2999(98)00518-4.

Peterhoff M, Sieg A, Brede M et al. (2003) Inhibition of insulin secretion via distinct signalling pathways in α2-adrenoceptor knockout mice. *European Journal of Endocrinology* 149, 343-350.

Pfeffer M, Smyth RD, Pittman KA et al. (1980) Pharmacokinetics of subcutaneous and intramuscular butorphanol in dogs. *Journal of Pharmaceutical Sciences*, 69(7), 801-803. doi:10.1002/jps.2600690715

Piascik MT, Kusiak JW & Barron KW (1990) α_1-adrenoceptor subtypes and the regulation of peripheral hemodynamics in the conscious rats. *European Journal of Pharmacology* 186, 273-278.

Pieters FA & Zuidema J (1986) Intra-adipose administration of monoacetyldapsone to healthy volunteers. *International Journal of Leprosy and Other Mycobacterial Diseases: Official Organ of the International Leprosy Association*, 54(4), 510.

Plummer JL, Crnielewski PL, Reynolds GD et al. (1990) Influence of polarity on dose-response relationships of intrathecal opioids in rats. *Pain*, 40, 339-347

Pypendop B, Serteyn D, Verstegen J (1996) Hemodynamic effects of medetomidine-midazolam-butorphanol and medetomidine-midazolam-buprenorphine combinations and reversibility by atipamezole in dogs. *American Journal of Veterinary Research*, 57(5), 724-730.

Pypendop BH (1998) Hemodynamic effects of medetomidine in the dog: A dose titration study. *Veterinary Surgery*, 27(6), 612-622. doi:10.1111/j.1532-950X.1998.tb00539.x

Pypendop BH, Honkavaara J, Ilkiw JE (2017) Pharmacokinetics of dexmedetomidine, MK-467 and their combination following intramuscular administration in male cats. *Veterinary Anaesthesia and Analgesia*, doi://doi.org/10.1016/j.vaa.2017.02.008

Rabin B, Guo T, Gregg K et al. (1996) Role of serotonergic neurotransmission in the hypnotic response to dexmedetomidine, an α_2-adrenoceptor agonist. European Journal of Pharmacology, 306, 51-59.

Raffe MR (1989) Classifying commonly used analgesic agents. *Veterinary Medicine*, 84(7), 687-690.

Rang HP. The receptor concept: pharmacology's big idea (2006) *British Journal of Pharmacology*, 147:9-16. doi: 10.1038/sj.bjp.0706457.

Restitutti F, Kaartinen MJ, Raekallio MR et al. (2017) Plasma concentration and cardiovascular effects of intramuscular medetomidine combined with three doses of the

peripheral alpha-antagonist MK-467 in dogs. *Veterinary Anaesthesia and Analgesia*, 44(3), 417. doi:10.1016/j.vaa.2016.04.006

Rolfe NG, Kerr CL, McDonell WN (2012) Cardiopulmonary and sedative effects of the peripheral a2-adrenoceptor antagonist MK-467 administered intravenously or intramuscularly concurrently with medetomidine in dogs. *American Journal of Veterinary Research*, 73(5), 587-594. doi:10.2460/ajvr.73.5.587

Ruffolo RR (1985) Distribution and function of peripheral α-adrenoceptors in the cardiovascular system. *Pharmacology, Biochemistry and Behavior*, 22(5), 827-833. doi:10.1016/0091-3057(85)90535-0

Ruwart MJ, Klepper MS & Rush BD (1980) Clonidine delays small intestinal transit in the rat. *Journal of Pharmacology and Experimental Therapeutics*, 212, 487-490.

Salla K, Bennett RC, Restitutti F et al. (2014a) A comparison in dogs of medetomidine, with or without MK-467, and the combination acepromazine-butorphanol as premedication prior to anaesthesia induced by propofol and maintained with isoflurane. *Veterinary Anaesthesia and Analgesia*, 41(2), 163-173. doi:10.1111/vaa.12094

Salla K, Restitutti F, Vainionpää M et al. (2014b) The cardiopulmonary effects of a peripheral alpha-2-adrenoceptor antagonist, MK-467, in dogs sedated with a combination of medetomidine and butorphanol. *Veterinary Anaesthesia and Analgesia*, 41(6), 567-574. doi:10.1111/vaa.12158

Sallinen J, Link RE, Haapalinna A (1997) Genetic alteration of α_{2C}-adrenoceptor expression in mice: influence in locomotor, hypothermic and neurochemical effects of dexmedetomidine, a subtype-nonselective α_2-adrenoceptor agonist. *Molecular Pharmacology* 51, 36-46.

Salonen JS (1989) Pharmacokinetics of medetomidine. *Acta Veterinaria Scandinavica Supplementum*, 85, 49-54.

Salonen M, Reid K, Maze M (1992) Synergistic interaction between alpha 2-adrenergic agonists and benzodiazepines in rats. *Anesthesiology*, 76(6), 1004-1011.

Salonen S, Vuorilehto L, Vainio O et al. (1995) Atipamezole increases medetomidine clearance in the dog: An agonist-antagonist interaction. *Journal of Veterinary Pharmacology and Therapeutics*, 18(5), 328-332.

Savontaus E, Fagerholm V, Rahkonen O et al. (2008) Reduced blood glucose levels, increased insulin levels and improved glucose tolerance α_{2A}-adrenoceptor knockout mice. *European Journal of Pharmacology* 578, 359-364.

Scheinin M & MacDonald E (1989) An introduction to the pharmacology of alpha 2-adrenoceptors in the central nervous system. *Acta Veterinaria Scandinavica Supplementum*, 85, 11-19.

Scheinin M & Schwinn DA (1992) The locus coeruleus site of hypnotic actions of α2-adrenoceptor agonists? *Anesthesiology*, 76(6), 873-875. doi:10.1097/00000542-199206000-00001

Scheinin H, Jaakola M, Sjövall S et al. (1993) Intramuscular dexmedetomidine as premedication for general anesthesia: A comparative multicenter study. *Anesthesiology*, 78(6), 1065-1075.

Schinkel AH, Smit JJ, Van Tellingen O et al. (1994) Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell*, 77(4), 491-502. doi:10.1016/0092-8674(94)90212-7

Schinkel AH, Wagenaar E, van Deemter L et al. (1995) Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *Journal of Clinical Investigation*, 96(4), 1698-1705. doi:10.1172/JCI118214

Schinkel AH & Jonker JW (2003). Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: An overview. *Advanced Drug Delivery Reviews*, 55(1), 3-29. doi:10.1016/S0169-409X(02)00169-2

Segal JL, Brunnemann SR & Gray DR (1988) Gentamicin bioavailability and single-dose pharmacokinetics in spinal cord injury. *Drug Intelligence & Clinical Pharmacy*, 22(6), 461-465. doi:10.1177/106002808802200603

Self IA, Hughes JML, Kenny DA et al. (2009) Effect of muscle injection site on preanaesthetic sedation in dogs. *Veterinary Record*, 164(11), 323-326.

Shapiro LE, Shear NH (2002) Drug interactions: proteins, pumps, and P-450s. *Journal of the American Academy of Dermatology*, 47(4):467-84

Sinclair MD (2003) A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. *The Canadian Veterinary Journal = La Revue Veterinaire Canadienne*, 44(11), 885-897.

Soldani G, Del Tacca M, Bernardini C et al. (1984) Evidence for two opposite effects of clonidine on gastric acid secretion in the dog. *Naunyn Schmiedeberg's Archives of Pharmacology*. 327,139-142.

Solhaug V Molden E (2017) Individual variability in clinical effect and tolerability of opioid analgesics - Importance of drug interactions and pharmacogenetics. Scandinavian Journal of Pain, 17:193-200. doi: 10.1016/j.sjpain.2017.09.009.

Stamatovic S, Keep R, Andjelkovic A (2008) Brain endothelial cell-cell junctions: How to "Open" the blood brain barrier. *Current Neuropharmacology*, 6(3), 179-192. doi:10.2174/157015908785777210

Stone LS, MacMillan LB, Kitto KF et al. (1997) The α_{2A} adrenergic receptor subtype mediates spinal analygesia by α2 agonists and is necessary for spinal adrenergic-opioid synergy. *The Journal of Neuroscience* 17, 7157-7165.

Sund RB, Schou J (1964) The determination of absorption rates from rat muscles: An experimental approach to kinetic descriptions. *Acta Pharmacologica et Toxicologica*, 21, 313-25.

Tanoue A, Koshimizu TA, Shibata K et al. (2003). Insights into α 1 adrenoceptor function in health and disease from transgenic animal studies. *Trends in Endocrinology & Metabolism*, 14(3), 107-113. doi:10.1016/S1043-2760(03)00026-2

Thurmon JC, Ko JC, Benson GJ et al. (1994) Hemodynamic and analgesic effects of propofol infusion in medetomidine-premedicated dogs. *American Journal of Veterinary Research*, 55(3), 363-367.

Thurmon JC, Tranquilli WJ, Benson GJ (2007). *Lumb and Jones' Veterinary Anesthesia*. 4th edition. Baltimore, Williams & Wilkins.

Vainio O, Vähä-Vahe T, Palmu L (1989) Sedative and analgesic effects of medetomidine in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, 12(2), 225-231. doi:10.1111/j.1365-2885.1989.tb00664.x

Vainio O (1990) Reversal of medetomidine-induced cardiovascular and respiratory changes with atipamezole in dogs. *Veterinary Record*, 127(18), 447-450.

Vainionpää M, Salla K, Restitutti F et al. (2013). Thermographic imaging of superficial temperature in dogs sedated with medetomidine and butorphanol with and without MK-467 (L-659'066). *Veterinary Anaesthesia and Analgesia*, 40(2), 142-148. doi:10.1111/j.1467-2995.2012.00768.x

van Herwaarden AE & Schinkel AH (2006) The function of breast cancer resistance protein in epithelial barriers, stem cells and milk secretion of drugs and xenotoxins. *Trends in Pharmacological Sciences*, 27(1), 10-16. doi:10.1016/j.tips.2005.11.007

van Oostrom H, Doornenbal A, Schot A et al. (2011) Neurophysiological assessment of the sedative and analgesic effects of a constant rate infusion of dexmedetomidine in the dog. *Veterinary Journal*, 190(3), 338-344. doi:10.1016/j.tvjl.2010.11.025

Vatinoxan hydrochloride - compound summary. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/182976 31.7.2019

Vickery RG, Sheridan BC, Segal IS et al. (1988) Anesthetic and hemodynamic effects of the stereoisomers of medetomidine, an α2-adrenergic agonist, in halothane-anesthetized dogs. *Anesthesia & Analgesia*, 67(7), 611-615. doi:10.1213/00000539-198807000-00001

Virtanen R & McDonald E (1987) Reversal of the sedative/analgesic and other effects of detomidine and medetomidine by MPV 1248, a novel a_2-antagonist. *Pharmacology and Toxicology*, 60, 73.

Virtanen R, Savola J, Saano V (1989) Highly selective and specific antagonism of central and peripheral a2-adrenoceptors by atipamezole. *Archives Internationales De Pharmacodynamie Et De Therapie*, 297, 190-204.

Vlaming ML, Lagas JS, Schinkel AH (2009) Physiological and pharmacological roles of ABCG2 (BCRP): Recent findings in Abcg2 knockout mice. *Advanced Drug Delivery Reviews*, 61(1), 14-25. doi:10.1016/j.addr.2008.08.007

Vracko R & Benditt EP (1970) Capillary basal lamina thickening. Its relationship to endothelial cell death and replacement. *The Journal of Cell Biology*, 47(1), 281. doi:10.1083/jcb.47.1.281

Vähä-Vahe AT (1990) The clinical effectiveness of atipamezole as a medetomidine antagonist in the dog. *Journal of Veterinary Pharmacology and Therapeutics*, 13(2), 198-205. doi:10.1111/j.1365-2885.1990.tb00769.x

Wakasugi H, Yano I, Ito T, et al. (1998) Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. Clinical Pharmacology and Therapeutics, 64(1), 123-128.

Yamaguchi I & Kopin IJ (1980) Differential inhibition of alpha-1 and alpha-2 adrenoceptor-mediated pressor responses in pithed rats. *Journal of Pharmacology and Experimental Therapeutics*, 214(2), 275-281.

Yáñez JA, Remsberg CM, Sayre CL et al. (2011) Flip-flop pharmacokinetics--delivering a reversal of disposition: challenges and opportunities during drug development. *Therapeutic Deliv*ery 2(5), 643–672. doi:10.4155/tde.11.19

Zuidema J, Pieters FA, Duchateau GS (1988) Release and absorption rate aspects of intramuscularly injected pharmaceuticals. *International Journal of Pharmaceutics*, 47(1-3), 1-12. doi:10.1016/0378-5173(88)90209-8